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The following courses of instruction will be given by the Liverpool School of Tropical Medicine during 1910:—

Full Course begins 6 January. Short Course begins 1 June.
Diploma Examination, 4 April. Certificate Examination, 29 June.
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The full Course of Instruction is open to all qualified medical men, and the Examination to all students who have taken out this full course.

Fee for the full Course of Instruction—Thirteen Guineas.

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For prospectus and further information, application should be made to the Dean of the Medical Faculty, University of Liverpool.

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1904	Greenidge, Oliver Campbell
1904	Hehir, Patrick
1904	Khan, Saiduzzafor
1904	Laurie, Robert
1904	Maclurkin, Alfred Robert
1904	McConnell, Robert Ernest
1904	Nicholson, James Edward
1904	Philipson, Nicholas
1904	Sharman, Eric Harding
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1906	Chisholm, James Alexander
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1906	Dundas, James
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1906	Jeffreys, Herbert Castelman
1906	Mackenzie, Donald Francis
1906	Pailthorpe, Mary Elizabeth
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1906	Sampey, Alexander William
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1906	Williamson, George Alexander
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1907	Bond, Ashton
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1907	Collinson, Walter Julius
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1907	Donaldson, Anson Scott
1907	Fell, Matthew Henry Gregson
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1907	Kenrick, William Hamilton
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1908	Le Fanu, Cecil Vivian
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1910	White, Maurice Forbes

EDITORIAL NOTICE

By order of the Committee of the Incorporated Liverpool School of Tropical Medicine, the series of the Reports of the School, which had been issued since 1899, were followed, from January 1, 1907, by the Annals of Tropical Medicine and Parasitology, of which this is the second number of the fourth volume.

Altogether twenty-one Memoirs, besides other works, were published by the School since 1899, and of these ten, containing 519 quarto or octavo pages and 95 plates and figures, were published during the two years 1904 and 1905.

The Annals are issued by the Committee of the School, and will contain all such matter as was formerly printed in the Reports—that is to say, accounts of the various expeditions of the School and of the scientific work done in its laboratories at the University of Liverpool and at Runcorn. In addition, however, to School work, original articles from outside on any subject connected with Tropical Medicine or Hygiene may be published if found suitable (see notice on back of cover); so that, in all probability, not less than four numbers of the Annals will be issued annually. Each number will be brought out when material sufficient for it has been accumulated.

PARASITIC GRANULOMA:
A CONDITION ALLIED TO ORIENTAL SORE OCCURRING IN EGYPT

BY

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AND

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(*Received for publication* 2 *April*, 1910)

PLATES XII-XV

Outpatient practice among the poorer classes in Egypt includes a variety of conditions not described in the English textbooks. One of these has presented itself with sufficient frequency to induce us to try and investigate it. It consists essentially of a chronic elevated patch or warty growth in the skin, sharply localised, and unaccompanied by other symptoms. Most of the cases we have so far seen have been structurally papillomata, others have consisted of flat skin-covered granulomata. We have described them as warty and flat forms respectively, and in both we have occasionally found 'bodies' of the same class as those described in Oriental sore. Clinically, however, these cases differ widely from the usual description of that condition, and we do not think they can be classed under that name as it is used at present.

Taking these two forms of the disease together, we have, so far, investigated clinically and pathologically ten cases of the nature of which we feel tolerably certain. Besides these, we have observed a number of similar ones in which some doubt existed, or of which full notes have not been kept. In addition to these, Professor Bitter and Dr. Dreyer, Bacteriologist and Assistant Bacteriologist respectively to the Egyptian Government, have each met with a case of a similar nature, in which the parasite was found. They have kindly permitted us to append their notes of

these cases to the present paper. We desire further to acknowledge our indebtedness to Professor Bitter for allowing us to reproduce his excellent micro-photographs of the parasites seen in Pl. XIV, figs. 11, 12, 13.

There is no doubt that this condition was equally common in the past, and the diagnoses show that there was less difficulty in distinguishing it from other diseases than in finding a suitable name for it. Among the names previously employed by various English and Egyptian members of the staff, we find 'papilliferous degeneration of skin'; 'chronic benign papilloma'; 'pseudo-epithelioma'; 'false elephantiasis'; 'hypertrophic dermatitis'; 'lupus erythematosus exanthematosus'; 'granuloma of foot'; 'partial ichthyosis'; 'parasitic growth'; 'fungous growth'; 'fungating granuloma.'

Cases bearing these very varied titles seem, as far as we can gather from reports, and in some cases observation, to have belonged to the class described above. These names are interesting as showing the clinical features which struck a number of different observers in individual cases.

Incidence. In the ten cases we have selected, the age of the patients varied from eighteen to sixty, with an average of thirty; they were all males, and with the exception of two students, all fellaheen. Five of them were affected on the forearm or hand, four on the legs or feet, one on the face. Half of them had single lesions, half multiple. The duration of the disease varied from six weeks to ten years; excluding the last figure they averaged six months.

Consequently, as far as we can conclude from so few cases, the disease, as we meet with it, is one affecting male fellaheen at any time in their adult working life, it may be single or multiple, and it affects the arms and legs about equally: the patients usually apply for relief about six months after the disease has begun.

We have never obtained any history of similar cases in the same village from which infection might have been acquired. Where infection is multiple, the lesions are usually near one another. This may be due to simultaneous infection, but more probably, especially where there is an interval of time between them, to auto-inoculation from scratching.

The chronicity of the condition is sufficiently well shown by the histories. The rate of progress varies a good deal in different cases, but is slow in all; the channel of infection is probably through the skin, for it appears almost exclusively on uncovered parts, i.e., the limbs and face rather than the trunk.

As regards the constitutional condition of the patients, they none of them gave any constant or important medical history, and their general health was clearly unaffected. In those cases in which we examined for enlargement of liver or spleen, it was absent.

I. WARTY FORM

These masses begin as small painless tubercles and may gradually attain a diameter of three or four inches. The skin round their edge is usually healthy, but it may be reddened from sepsis, or show a definite areola marked by loss or increase of pigment. They have a very definite, raised, even overhanging edge, and may rise nearly an inch abruptly from the level of the surrounding skin. The surface of the growth, by the time it is seen, is often ulcerated in whole or part, but typically is covered by hard whitish epithelium, and presents a warty cauliflower-like appearance. It is intersected by clefts lined with foul decomposing epidermis; indeed, where bacterial infection has occurred, it is possible to express masses of dead epithelium with pus from numerous apertures under the overhanging edge. In places where movement naturally takes place, as on the front of the ankle, some of these clefts deepen and appear as transverse fissures which extend through the skin and give rise to pain on movement. With this exception, the disease appears to be painless. There is occasionally some local oedema and enlargement of lymphatic glands, but these depend on secondary sepsis and are not an essential part of the disease.

Most of these growths become septic in course of time, and full of mixed infection. Most of their offensive smell and foul appearance is due, however, to decomposition of epithelial masses on the surface and in the clefts, outside the substance of the growths altogether. If they could be kept clean, which of course

they never are, they would probably show a firm white mammillated surface, like the head of a raw cauliflower, to sight and touch.

A very striking feature, and one that marks them off at once from malignant growths, is the way in which they are confined to the skin, and hardly ever affect even the most superficial tendons. An extensive growth on the dorsum of the foot scarcely affected the movement of the extensors of the toes. And it is quite easy, after running an incision round them, to strip them like a scalp off the deep fascia, leaving a smooth surface on which grafts take readily.

An exception to this was Case VIII, in which the growth situated on the inner side of the elbow, had enveloped the ulnar nerve and invaded the internal condyle for a short distance. But this growth had been present for ten years, and the ulnar nerve, which ran through it much as the spinal accessory runs through tubercular glands, was easily freed, and covered with a skin graft. It retained its motor and sensory functions unimpaired. The fact that the growth could completely surround a nerve for a distance of some two inches for a period of several years, and leave it functionally active and sufficiently well nourished to take a skin graft on its surface, is very good evidence of its sharp limitation and innocent character.

II. FLAT FORM

So far we have only seen two instances of this variety as against eight of the preceding. They both occurred in students of the higher schools, one on the face and one on the forearm, and had been present for four and twelve months respectively. In appearance they were flat pink patches, raised one or two millimetres above the surrounding healthy skin, covered with thin epidermis, sharply limited, painless, soft, and freely moveable on the deeper structures.

Their essential identity with the preceding form is suggested by the fact that they contained similar intracellular parasites. It is possible that the apparent differences are due to external causes. Both these cases were in men of the educated class, who kept the growths clean and protected from irritation. It is possible that, if

they had been situated on the bare and dirty limbs of fellaheen, exposed to constant friction and bacterial invasion, they might have shown the same proliferative changes which are so marked in the papillomatous form.

The two cases recently described by Balfour* fall into this class, in which, in our experience, the parasites are present in much larger numbers.

Treatment. A very large variety of lotions and ointments have been used for these growths. They seem to have no effect, beyond slightly diminishing the sepsis.

In one case a determined attempt was made to treat a large patch by ionization with iodine. Under this treatment there was distinct improvement up to a certain point, and the size of the growth was measurably lessened; but after six weeks there was not enough change to make it worth while continuing.

For fellaheen especially—who cannot afford to spend a long time in hospital—the only treatment worth considering is that of excision of the whole mass, followed by immediate skin grafting. The growths can be readily stripped off the underlying fascia, and in spite of the sepsis usually present, grafts take fairly well. A good example of this is shown in Pl. XIII, fig. 8 (Case VIII).

CASES

Case I. Pl. XII, figs. 1, 2. Man aged eighteen, admitted January 21, 1907, under Mr. Richards. Six weeks ago he had swelling of both legs; a few days later small red patches appeared, which increased in size and turned brown.

On admission there is a brown circular raised patch, three inches across, divided by irregular furrows containing a yellowish-brown secretion, situated in front of the ankle and extending back past the external malleolus. A similar one on the internal malleolus of the right leg, and a smaller one above it. Two more on the dorsum of the left foot. The patches have a foul smell, and are surrounded by an inflamed area. There is a little oedema of both feet.

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* 'Trans. Soc. Trop. Med. and Hyg.,' Vol. III, No. 3, p. 107, January, 1910.

The feet were cleaned, and various patches were treated with salicylic, boric, and yellow oxide of mercury ointment respectively. Potassium iodide was given internally.

After twelve days in hospital there was no improvement, and he was discharged at his own request, unrelieved.

Pathological Report (A.R.F.)

Great overgrowth of epithelium, with a cellular and vascular granulation tissue, pushing its way upwards through and between the greatly hypertrophied papillae. No purulent process or appearance suggestive of Madura foot. It may be a simple chronic wart, but the character of the infiltrating tissue beneath reminds one of a specific infiltration.

Case II. Pl. XIII, fig. 6. Man aged thirty, admitted August 4, 1908, under Mr. Richards. Four months ago a small painless papule, without discharge, appeared on the outer malleolus of the left leg, and increased in size.

The mass is the size of an egg, oval, raised above the surface, hard, papillomatous. The small papillae are of a purplish colour, and between them the surface is covered by yellowish crusts. It is sensitive to touch, but not painful, even on pressure.

Similar smaller growths occur on the chest and the right second toe, dating two and four months respectively.

It was treated for 18 days with liniment and tincture of iodine, and showed great improvement, but did not disappear. The patient then left hospital at his own request.

Case III. Pl. XII, figs. 3, 4. A man aged twenty-five, admitted April 23, 1908, under Dr. Phillips, transferred to Mr. Richards April 29.

Ten months ago he noticed rough, red, warty, painless masses appearing in front of right ankle and on dorsum of left foot. As they grew older they became white and encrusted. Four months later they began to be painful. No similar case in the district.

Right foot. A square papillomatous mass 12 × 11 cm. raised 2 cm. from the surface, over the front of the ankle joint. It is divided by *three deep transverse clefts* extending *right through it*, and filled with foul-smelling epithelium. The surface is covered with

epidermis, and has a low-set warty cauliflower appearance. There is no ulceration. It is surrounded by a margin 1-3 cm. broad, in which the skin is raised, smooth and glistening with some loss of pigmentation.

A similar oval patch 7 × 3 cm. is found over and behind the external malleolus. Movement of tendons unaffected.

Left foot. A similar patch, 12 × $7\frac{1}{2}$ cm., covering base of four inner toes and adjacent dorsum. Another over tendo Achillis, 6 × $4\frac{1}{2}$ cm., its lower margin level with malleoli. This has no cracks. Enlarged glands in groin on both sides.

Pathological Note (A.R.F.)

A case, in my opinion, of the same parasitic nature as the two others (I, II). The parasite may be:—*a*, a pathogenic yeast; *b*, a mycelial fungus, Botryomycosis.

Treatment. All the growth was stripped off the right foot, leaving a smooth grey surface, which was painted with iodine, and afterwards skin-grafted. It healed well, but in July small warty indurations began to form in the scar, suggesting recurrence.

The left foot was treated by ionization with iodine. It decreased in circumference from 27 to $25\frac{1}{2}$ cm., became painless and the movements of the toes quite free. It then remained stationary.

He was discharged at his own request, July 11.

Case IV. Pl. XIII, fig. 5. A man of 38, admitted April 24, 1909, under Dr. Day for cirrhosis and ascites.

On the right hand is a growth extending over the metacarpals of the first three fingers, with a raised border. The lesion was of a year's duration. It presented itself as a circular area with a firm, raised edge, which was rounded, covered with skin, and painless. Manipulation of the lesion was freely permitted, although the man said that it sometimes pained him at nights. There was no ulceration to speak of. The centre consisted of a thin, wrinkled cicatrix, said to be due to cauterization. The lesion had commenced centrally, and extension had since been going on at the edges.

Naked-eye section of the margin showed it to be composed of a solid white mass of epithelium, the processes of which ran

downwards in parallel fashion towards a very definite lower boundary.

Pathological Report.

Repeated search in films failed to show Leishman-Wright parasites. Bacteria were not infrequently met with. They were diplococci, very like pneumococci, and some short bacilli. Histologically it was a sore of papillomatous type.

Case V. A man aged 21. Admitted on March 17, 1909, under Mr. Richards. No venereal history in self or family; no similar growths in the village; has inquired from everyone.

The present masses appeared six months ago, and have been painful enough to prevent his working for the last three months.

On the left foot is a patch, three inches by two, on the outer side of the front of the ankle, raised a quarter of an inch from the surface, partly skin-covered, partly granulomatous, surface like a cauliflower, definite sinuous raised edge surrounded by healthy skin.

A second similar patch, four inches by two, rising abruptly half an inch from healthy skin, starts at the front border of the tibia, and goes round past the tendo Achillis. The edge of the growth overlaps the surrounding skin. Both masses have yellow scabs adherent to their surface in places, and smell foul. Inguinal glands enlarged, foot and leg oedematous. The growths were stripped off by Dr. Aly Bey Ibrahim, and immediate skin-grafting done.

Patient discharged cured soon afterwards.

Pathological Report (A.R.F.)

The tissue is essentially of papillomatous structure, the penetrating epithelial columns passing into an almost continuous zone of small-celled infiltration. On careful examination, this proves to be mononuclear in character. In films treated by Giemsa's stain, large numbers of bodies having the closest resemblance to the parasite of Kala-Azar (*L. donovani*) but

doubtless of the species *L. tropica* are found, both free and in the interior of large mononuclear phagocytes.

Case VI. A man aged 60, admitted August 23, 1909. A year ago a swelling appeared on the forearm, near the wrist, and four months ago a smaller one came higher up.

Two rough raised patches with thick everted edges, covered with scabs, and containing maggots. They were excised, and he was discharged cured.

Pathological Report.

No Leishman-Wright parasites found in scrapings. A good deal of mixed bacterial invasion, which may account for their absence. I have no doubt the growth is a parasitic papilloma.

Case VII. A man aged 26, admitted March 31, 1909, under Mr. Richards. Nine months ago a number of small red lumps appeared near one another on the inside of the middle of the left forearm.

The growth is superficial, does not affect movements. Its edge and part of its surface are covered with skin. Treated by excision and grafting, and discharged cured April 24.

Pathological Report.

Search for parasites, so far, negative. A rich secondary microbial infection, which adds to the difficulty of the search. The margins are diffusely infiltrated with pus.

Second Report. Prolonged examination revealed the presence, in the films, of the same parasite (*Leishmania tropica*) as was discovered in a preceding case (No. V), but in very scanty numbers. These were only found free amongst the bacteria of secondary invasion. Although their form was identical with that of those previously found, the staining of their cytoplasm, and in particular that of their larger chromatin masses was defective.

Case VIII. Pl. XIII, figs. 7, 8. A man aged 45, admitted October 27, 1909, under Mr. Richards. His trouble began as a small boil ten years ago, and has increased since. No treatment. No similar cases in the village.

The right arm is occupied from the elbow to half-way up the humerus, and over the inner and front half of its circumference by a swelling, rising with an abrupt and overhanging edge from the skin to a height of $1\frac{1}{2}$ to 2 cm. The skin immediately round the edge has a dark pigmentation about 2 cm. broad, but is otherwise healthy. The surface of the growth is mammillated, with ulcerated patches where the skin has worn off the tops of the projections, and divided by skin-lined cracks containing a foul fluid. The upper part moves freely on the deep tissues, the lower is anchored to the condyle. The arm is fixed at an angle of 85°, with scarcely any flexion or extension, but pronation and supination are free. No glands in the axilla. At the operation the upper part of the growth peeled easily from the deep fascia, lower down it surrounded, without invading the ulnar nerve, and involved the bone just above the internal condyle. This area—the only one where there was any deep invasion—was scraped out and packed with sulphur; the remaining surface was grafted; and in spite of the filthy condition of the arm, most of the grafts took well. The ulnar nerve, which lay bare and isolated beneath the grafts, preserved all its motor and sensory functions. He was discharged with a small sinus leading to the bone.

Pathological Report (A.R.F.)

Microscopical search in films, treated with Giemsa's stain, shewed no *Leishmania tropica*. Very numerous bacteria, diplococci, short streptococci, and an undetermined species of bacillus, were present. These, when present in large numbers, seem to determine the disappearance of the parasite.

Case IX. Pl. XV, fig. 9. A third year medical student, dressing for Mr. Richards, complained of two patches, one on his forearm, the other on his wrist, slightly elevated, flat, soft, pinkish, skin-covered, sharply limited, and freely movable. They had been present about a year. They were both excised under cocaine-adrenalin anaesthesia.

Pathological Report (A.R.F.)

The skin over the central portion of the lesion shows only slight thickening. There is beneath a dense, almost homogeneous mononuclear cell-infiltration; in two places, degenerated foci occur, in which numerous remains of cell nuclei are visible, but no bacteria. Films were not made from this case, but the characteristic parasite was found in sections, though not plentifully, in the larger mononuclear cells of the subcutaneous infiltration.

Case X. A student of the School of Law was admitted under Mr. Richards, with a patch on the cheek immediately below the left eyelid, measuring one inch by five-eighths. Flat, soft, pink, smooth surface, covered by thin unbroken skin. The skin around was quite healthy. It had been present for the last four months, and had gradually increased in size.

It was excised by Dr. Aly Bey Ibrahim.

Pathological Report (A.R.F.)

The specimen, as received, was incised by parallel sections, and placed in Formol-methyl-alcohol; microscopical sections being stained with Haem-alum and Eosin and also by Giemsa.

The skin over the lesion was much reduced in thickness, the majority of the Malpighian papillae having disappeared. The roots of hairs and sweat glands had also largely disappeared in an exceedingly dense cell-infiltration, which penetrated the corium for some distance. This, on examination with higher powers, was found almost exclusively mononuclear in character, the majority of the cells being of small size. Paler areas of larger mononucleated elements, however, were present as rather definite nodules in the midst of the small-celled infiltration. These were found filled with enormous numbers of parasites, very few occurring outside the cells. They were not so plentiful immediately under the skin.

Case XI. Note by Dr. Bitter, October, 1908. The case was that of a native officer of the Egyptian Army in Cairo, who had never been out of Egypt. He had small disseminated tumours, elevated about two to three millimetres, which were not ulcerated and covered with dry, whitish-coloured scabs

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of epithelium. The diameter of the individual tumours varied from two to ten millimetres. About twenty of these tumours were found on the right forearm from the elbow down (Pl. XV, fig. 9), three on his face, one on the forehead and two in the angle of the nose. He had consulted Dr. Scheuber, a dermatologist in Cairo, who first thought it might be a case of leprosy, and who sent the patient to me for bacteriological examination. The research for leprosy bacilli was, however, negative; but I found the Leishman-Wright bodies in smears as well as in sections of an excised tumour. The parasites were situated in big mononucular cells (macrophages) which were abundant in the stratum just underneath the epithelium.

The same dermatologist brought, a few months later, some slides and an excised tumour to Dr. Dreyer, telling him that they had been taken from a Roumanian lady who had lived in Cairo for several years, and who was suffering from a similar affection on the face and neck. The size of the tumour was, however, bigger. Her sister was said to suffer from the same affection. Dr. Dreyer found a good many Leishman-Wright bodies in cells of the same type as in the case of Dr. Bitter.

*Case XII. Note of a case by Captain M. F. White, I.M.S.** (Pl. XV, fig. 14). The papules were first noticed in February, 1909, in Bushire (Persian Gulf), where cases were scarce at the time, none of the Europeans being affected. Had been in Baghdad two months previously.

The 'flea-season' was on in February and not much notice was taken of the spots. They consisted of a group of five small spots on the dorsum of foot, and two others further up about an inch away were noticed later. They varied in diameter from 3 to 5 mm. They were slightly raised with a dull red periphery, with a small scab in the centre, raised in some, depressed in others. The whole area of the five spots was slightly raised and inflamed. They gave rise to no symptoms, pain, or trouble whatsoever, except that occasionally after a hot bath they gave rise to slight tingling and became slightly swollen and red. Otherwise they have shown no sign of increasing or decreasing, and at the present time, fourteen

* We beg to express here our thanks to Captain White for his note and photograph, and to the Authors for permission to include it in this paper.—EDS.

months after they were first noticed, they are practically in the original condition.

For the last month, two of the spots which have been used for microscopic examination, have taken to remaining open for a few days, but always heal up again with the aid of a simple dressing.

Smears were taken in April, 1910, and typical parasites (*Leishmania tropica*) found. None were found in the peripheral blood, though many attempts were made to find them.

Cultures were tried twice on sodium citrate, acidified with citric acid, and incubated at 22° C. according to Rogers' method, but no result was obtained. Cultivation was then tried on blood agar, with positive result in each tube inoculated. The cultures showed *Herpetomonas* forms after two days' incubation, but it was found impossible to maintain them and to make subcultures owing to the contaminating micrococci of the skin.

PATHOLOGICAL HISTOLOGY OF THE LESIONS

It may at once be said that the pathological features of the two types above described present an underlying identity. The differences between them, although very great, both as regards their naked eye and histological characters, are, we believe, explicable on the ground of accidental influences depending on situation, exposure to friction, bacterial invasion, etc. The essential identity of the two forms is indicated not only by the presence in each of indistinguishable parasites, but by the essentially similar nature of the sub-epithelial infiltration which both present.

Warty Form. So far, we have only observed the warty forms in exposed situations where the skin is normally of considerable thickness, and where such growths are liable (in the absence of any protective covering) not only to bacterial invasion, but also to proliferative changes consequent upon the maintenance of a chronic inflammatory condition from irritative influences of a diverse nature.

In a thin paraffin section including the elevated margin of the lesion, one sees a number of vertical epithelial columns which penetrate the dermis sometimes to a depth of 7 to 8 mm. (Pl. XIV, fig. 10).

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In a thin paraffin section including the elevated margin of the lesion, one sees a number of vertical epithelial columns which penetrate the dermis sometimes to a depth of 7 to 8 mm. (Pl. XIV, fig. 10).

Small, apparently isolated down-growths of epithelium, the

cells of which have a normal arrangement, also occur in the deeper lying tissue. Structures resembling 'cell-nests' have been frequently observed both in the epithelial columns and in the deeper epithelial collections just mentioned. The 'prickle-cell' nature of the epithelial down-growths is, as a rule, very clearly seen, the intervals between the cells being rather greater than usual by reason of inter-cellular oedema. The stratum granulosum and the keratinous layer are usually considerably increased in thickness. These features of the epithelium are simply those which might be expected to result from any chronic lesion. The 'cell-nests,' for instance, have no greater significance than they possess in other chronic lesions such as simple warts, scrofuloderma, etc.

The dermis itself is the seat of an exceedingly dense and uniform cellular infiltration which is continuous beneath the entire extent of the lesion. This infiltration is composed of cells of various kinds. The superficial strata of the dermis in the central parts of the lesion frequently contain considerable numbers of polymorphonuclear leucocytes, as well as free nuclei and other cellular detritus, the result of inroads by pyogenic bacteria. More deeply, however, the cellular infiltration is composed almost exclusively of mononuclear elements of various kinds. A certain proportion of these are indistinguishable from lymphocytes. In the midst of this cellular zone, small ill-defined areas are observed composed of mononuclear cells of much larger size than the preceding class. Such areas, seen under a low power, appear rather paler than the dense small mononuclear infiltration surrounding them. The parasites are found in the largest numbers in the interior of the cells composing such areas, though by no means exclusively confined to them. The infiltrated areas just described are fairly vascular, considerable numbers of small vessels of capillary character being present.

Hair-follicles and sweat-glands, which are often of course encountered, share to some extent in the surrounding infiltration, but have not been observed to suffer any degenerative or destructive change.

Deeply, the infiltrated zone gradually merges into the normal areolar tissue proper to the site.

Flat Form. The skin here, in contrast to the preceding form, is thinned and atrophied, the glands of the skin sharing markedly in the atrophic process. The papillae of the Malpighian layer are represented by very short processes, or are at the centre of the patch, entirely absent.

A sharply defined zone of cellular infiltration, practically identical in all respects with that described as pertaining to the warty form, is present under the thinned epidermis. Such a growth, examined in section with a pocket-lens, closely resembles the subcutaneous nodules sometimes seen in cases of leucocythaemia. The parasites, which occur in the large mononuclear cells described above, were present in such large numbers in both cases of this kind which we have examined, that the sections appeared to be crowded with them.

The parasites agree in every respect with the descriptions of *Leishmania tropica*. They are identical in both the warty and flat forms of the lesion (Pl. XIV, figs. 11, 12, 13).

Our two examples of the flat form were entirely free from micro-organisms. In the warty forms, on the contrary, these were constantly present. These micro-organisms were of many different kinds, and as they clearly represented a secondary accidental infection, we have taken no pains to determine their nature. They were most frequent in the superficial layers, and were never found deeply in the centre of the growth.

The examination of a large number of specimens has shown that in those cases in which bacteria were most plentiful, parasites occurred in very scanty numbers or not at all. Our opinion is that, so far from there being anything of the nature of a symbiosis between the two, the appearance of bacteria involves the extinction of the parasite. The reason why we have failed to find the parasites in so many of the cases reported, is probably that this process of extinction had reached a point where exceedingly few, if any, parasites had survived. The specimen which contained the parasite in largest numbers was one which contained no bacteria. Whether the bacteria ever destroy the parasites sufficiently to bring about a natural cessation of the process we have no means of judging, but it seems quite possible.

The condition is therefore essentially one in which the

subdermic tissues are invaded by *Leishmania tropica*. Almost certainly, the parasite, after its entrance has been effected, multiplies in enormous numbers. The large non-granular mononuclear cells are those which are primarily attracted to the site of infection, and they harbour the parasite in large numbers in their interior. Whether intracellular multiplication of the parasite occurs or not, we have no means of stating with certainty, but it would appear probable.

The skin over the site of infection may or may not show hypertrophic changes; in other words, the resulting lesion may be either a prominent centrally ulcerated papilloma, or a slightly elevated non-ulcerated patch. In either case, the essential nature of the underlying infiltration is the same. We have, unfortunately, not been able to make any inoculation or cultural experiments with the parasite.

The facts to which we have called attention may be thus summarised:—

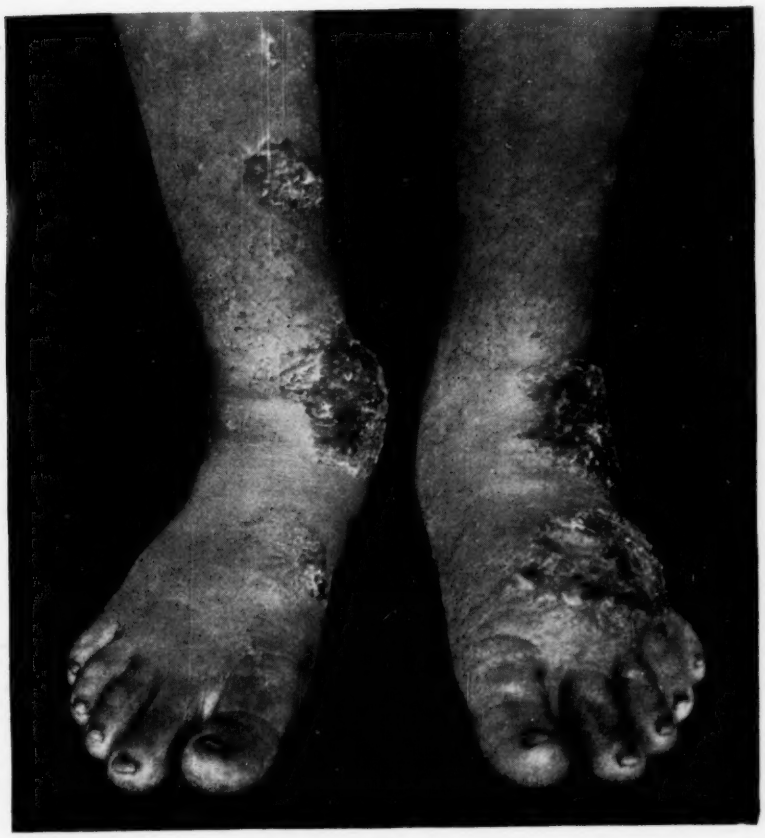
(1) Certain forms of skin affection caused by *Leishmania tropica* occur not infrequently in Egypt.

(2) They may be solitary or multiple, and in the latter case are almost certainly the result of auto-inoculation.

(3) They consist essentially of a mononuclear infiltration of the subcutaneous tissues which harbour, sometimes, large numbers of the parasites.

(4) The lesions manifest themselves clinically under two forms: the one, a slightly raised, smooth, flat patch; the other, a prominent warty growth. They run a chronic course, and are unaccompanied by constitutional disturbance.

(5) They are best treated by excision and immediate skin-grafting.



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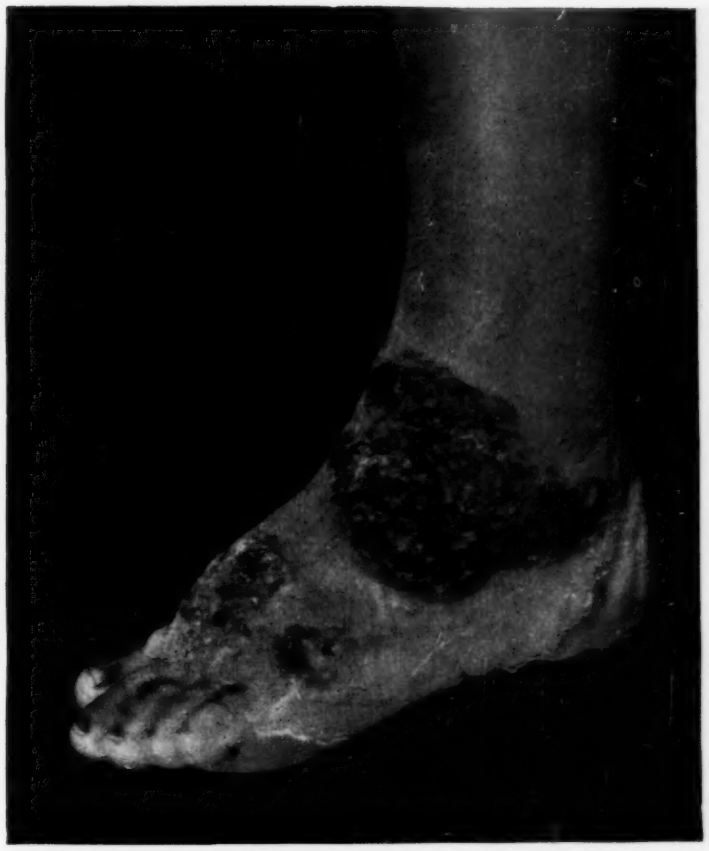

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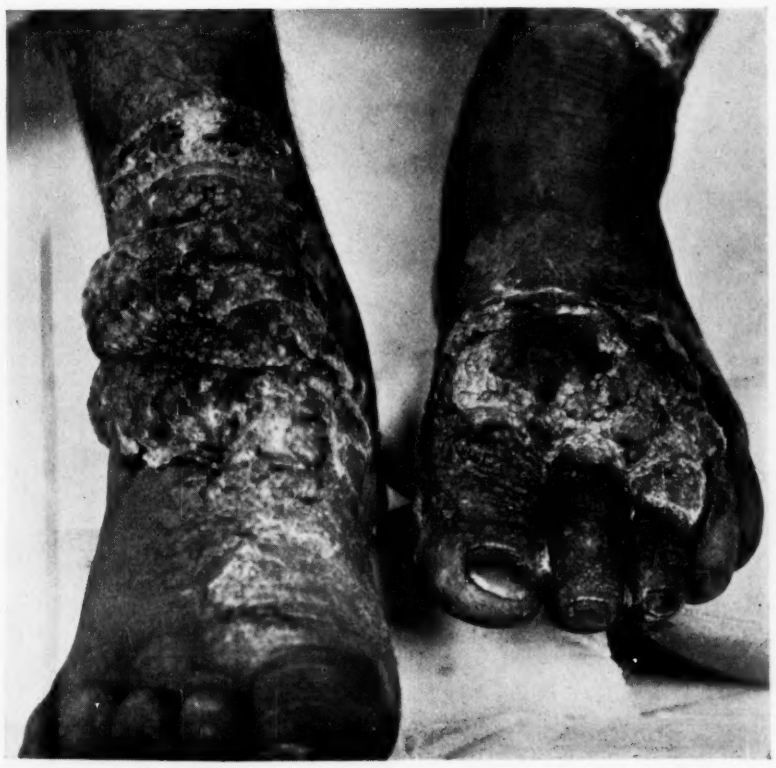

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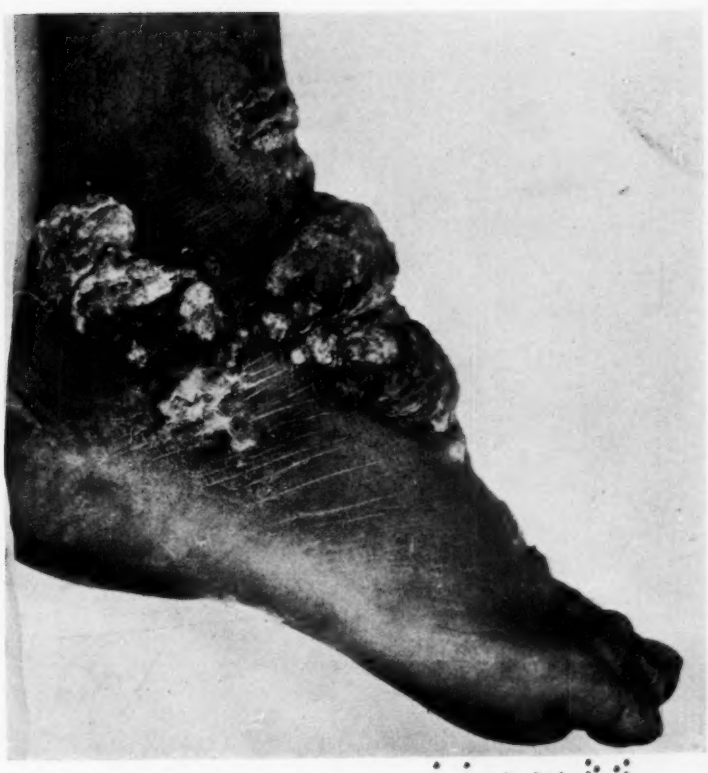

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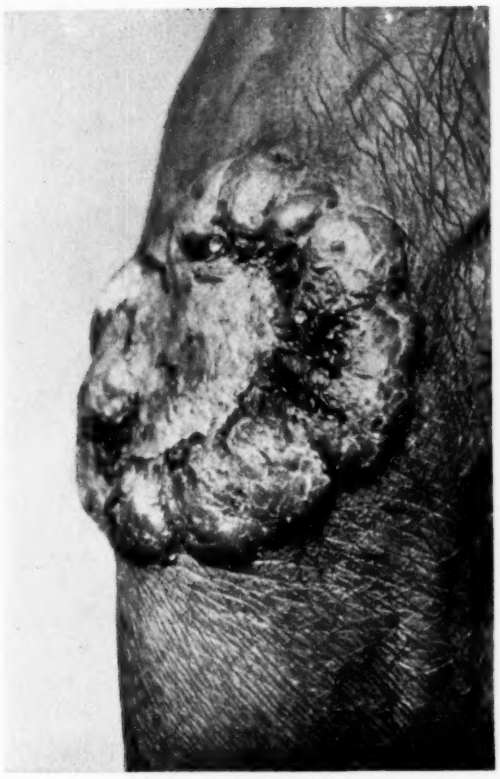

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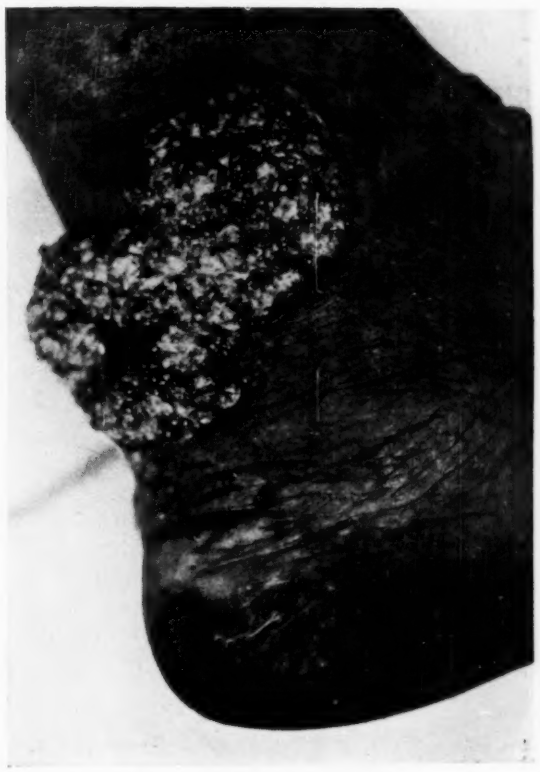

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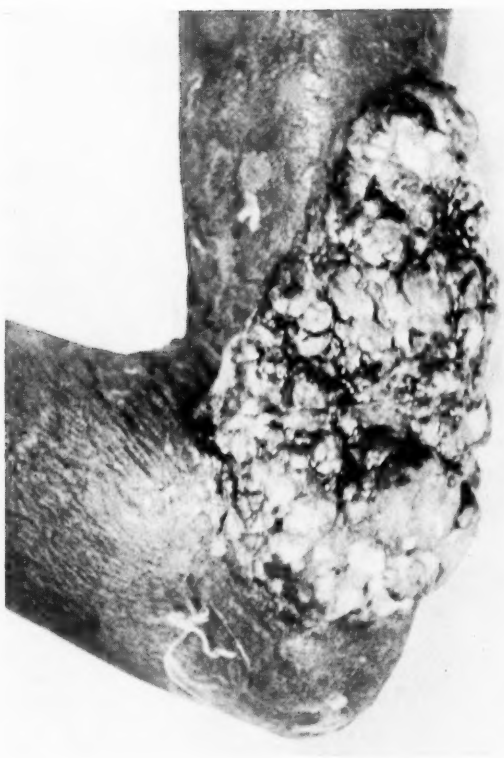

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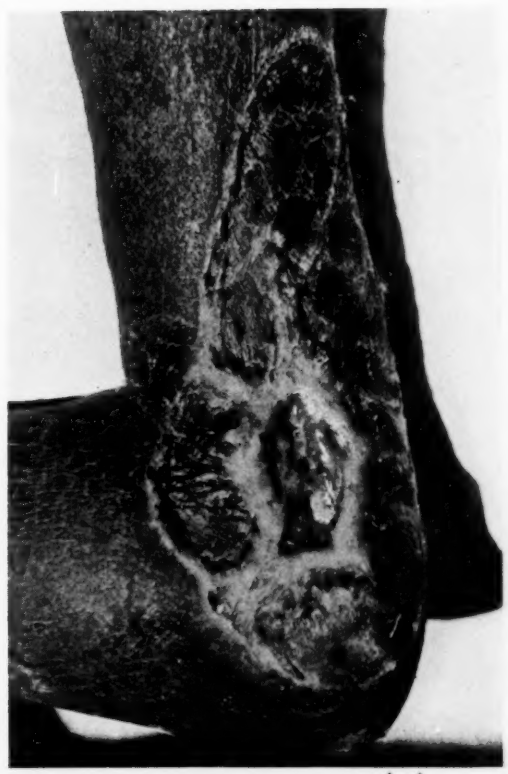

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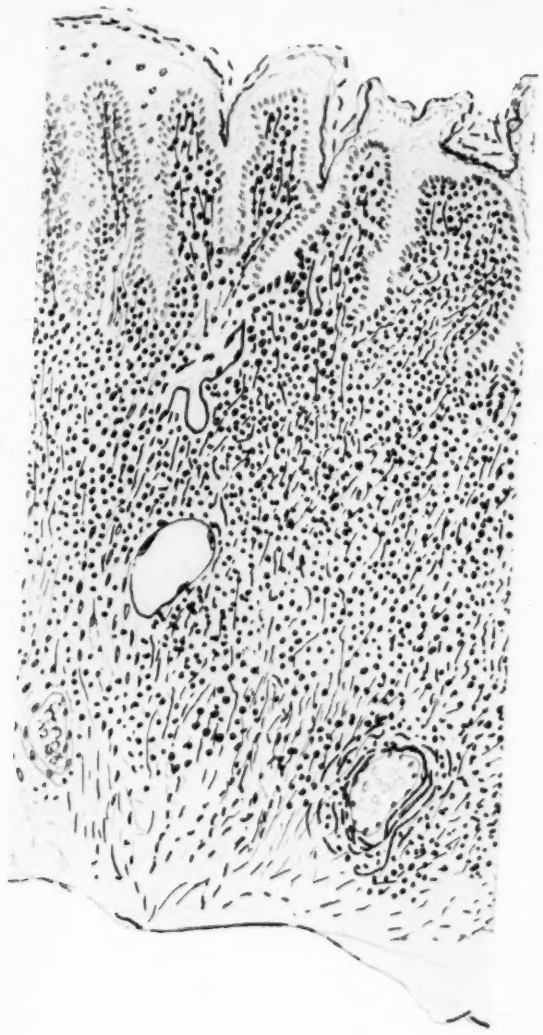

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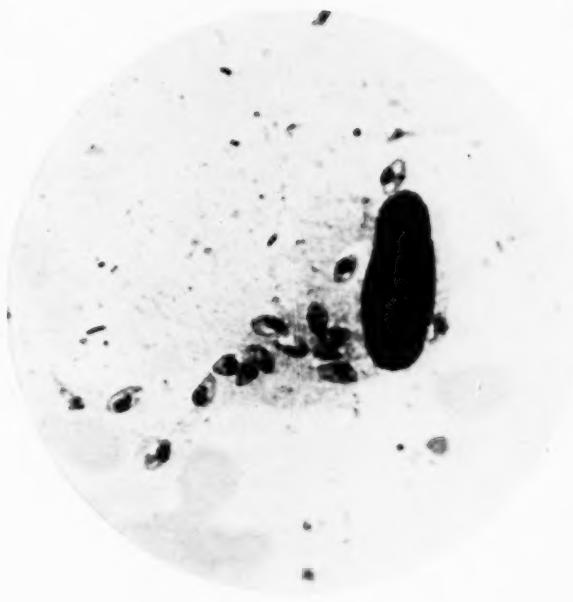

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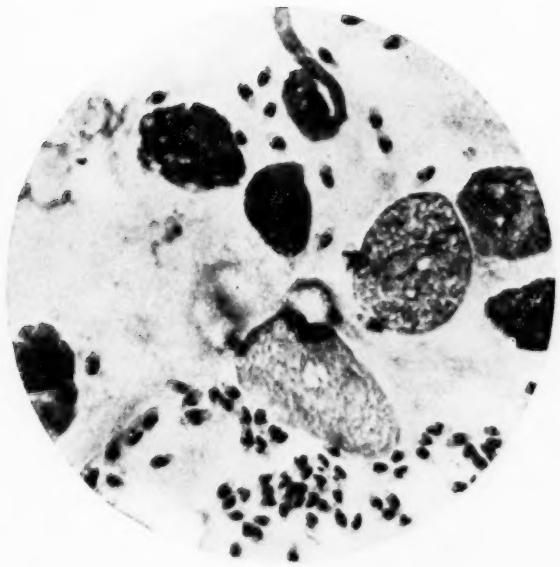

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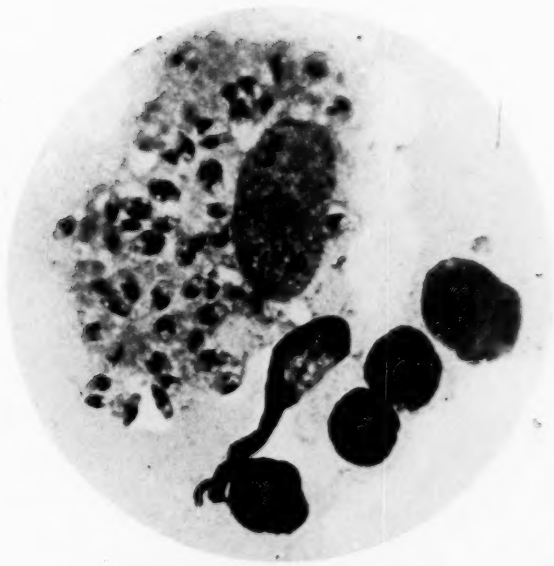

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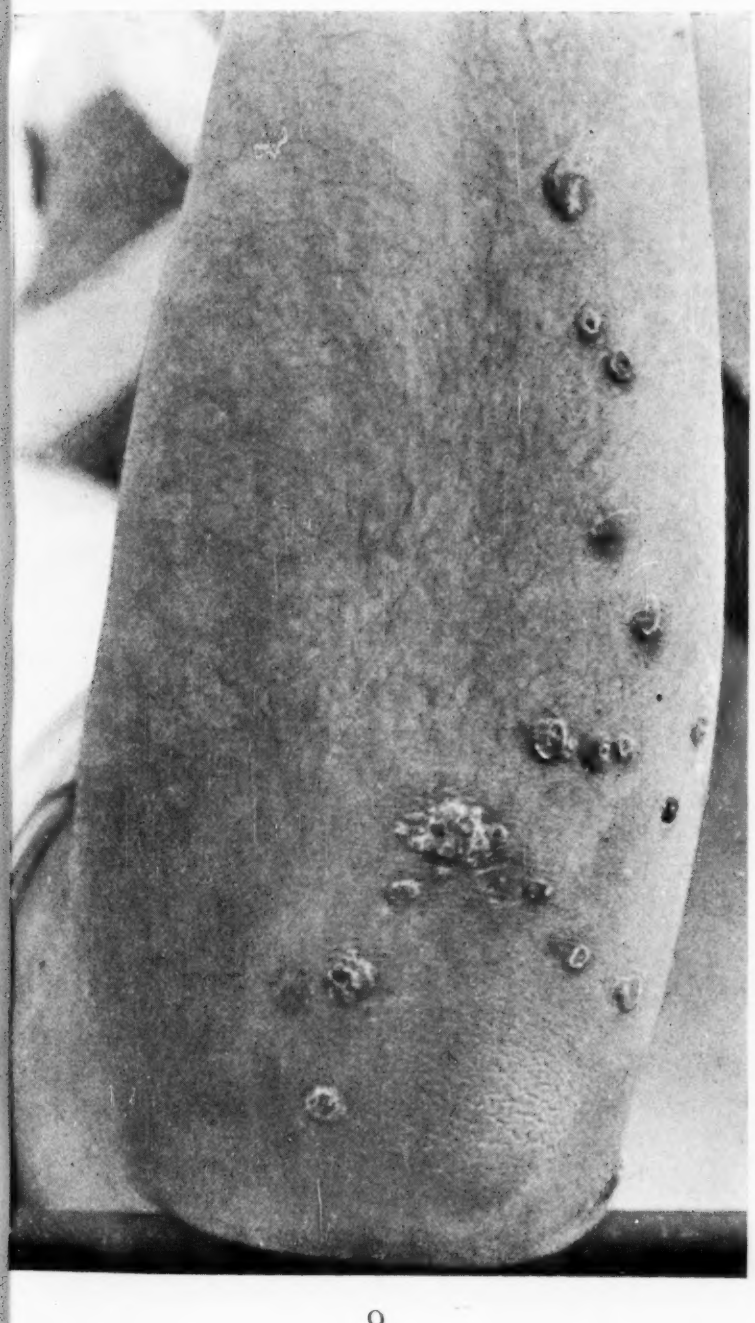

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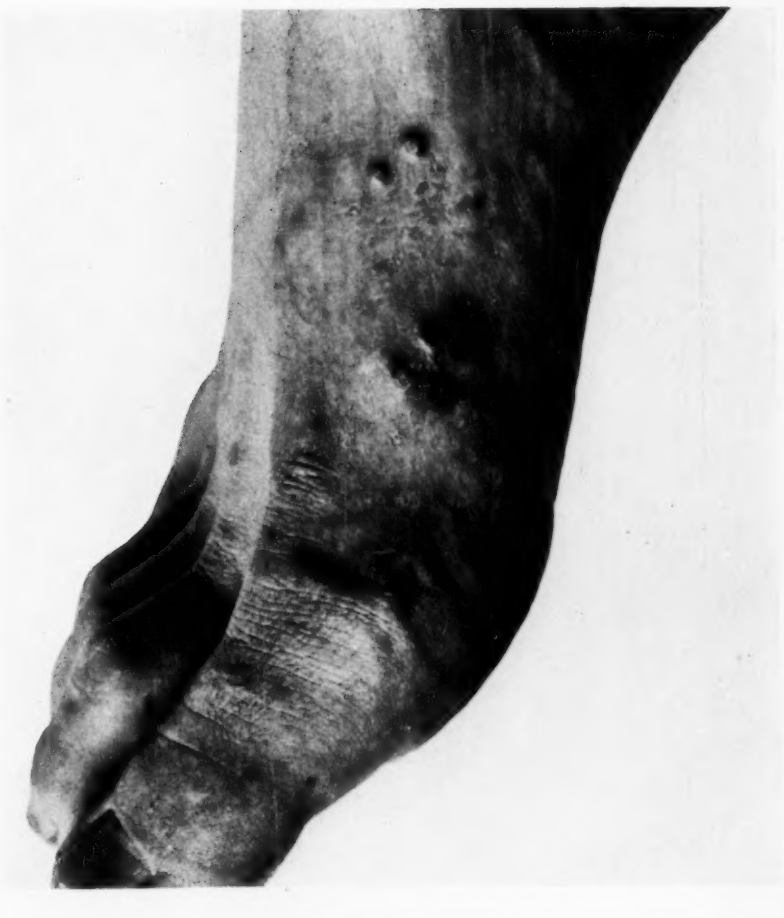

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CONTRIBUTION A L'ETUDE DU *POROCEPHALUS ARMILLATUS*

PAR

A. BRODEN

ET

J. RODHAIN,

Laboratoire de Léopoldville (Congo Belge)

(*Received for publication 5 April, 1910*)

Dans deux communications précédentes[1] parues dans ces *Annals*, Vol. I, No. 4, 1908, et Vol. II, No. 4, 1909, nous avons relaté des cas d'infection naturelle par *Porocephalus** chez l'homme, le singe, et certains grands serpents; ensuite nos expériences d'infestations expérimentale.

La présente notice à pour but de compléter certaines observations déjà signalées précédement.

I. INFESTATIONS EXPÉRIMENTALES DE L'HÔTE DÉFINITIF

On aurait pu objecter à nos expériences d'infestation expérimentales de l'hôte définitif, relatées dans ces *Annals*, Vol. II, page 311, que nos petits serpents, *Causus rhombeatus*, pouvaient être infectés naturellement. Cette Hypothèse nous paraissait inadmissible, car, malgré le grand nombre de ces *Causus* examinés par nous à Léopoldville, aucun n'avait été trouvé porteur de *Porocephalus*, analogue à ceux servant à nos expériences. Néanmoins nous avons repris ces essais d'infestation expérimentale, en essayant de suivre de plus près le sort des larves de Porocephalus chez le serpent en expérience; ensuite en donnant aux serpents un nombre déterminé de larves, à comparer au nombre de parasites trouvés ultérieurement à l'autopsie.

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* D'après une lettre privée que vient de nous envoyer le professeur Gedoelst, ces communications, ainsi que la présente, traitent en réalité de *P. armillatus* et non pas de *P. moniliformis*.

Le 25-IX-08. Nous tuons le singe No. 5 *Macacus*, infesté le 27-IV-08, c'est-à-dire, 5 mois auparavant, avec des œufs de *Porocephalus* du serpent No. 2 (*Python sebae*) (Voir ces *Annals*, Vol. II, page 307).

Ce singe est fortement infesté par des larves enkystées, d'une façon analogue à celle décrite pour le singe No. 2 (ibid. pages 306 et 307).

Quelques unes de plus grosses larves de ce singe No. 5, servent à l'alimentation forcée de 3 petits serpents.

Serpent No. 1. *Causus rhombeatus*, reçoit le 25-IX-08 plusieurs larves. Celles-ci doivent être introduites au fond du pharynx, pendant que l'animal est maintenu. Le nombre de larves introduites n'a pas été compté.

Le 28-IX-08 au matin ce serpent est trouvé mort. A l'autopsie, nous trouvons des suffusions hémorrhagiques le long de la moitié antérieure du tube digestif, jusque vers la partie postérieure de l'estomac.

Dans la cavité stomacale, nous trouvons 2 larves sorties de leur membrane kystique, adhérant par les crochets à la paroi de l'estomac. 2 autres larves, non sorties de leur enveloppe et mortes, sont contenues dans la cavité stomacale. Dans le tissu conjonctif au niveau de l'estomac, mais en dehors de celui-ci, 2 larves libres.

Dans les sacs pulmonaires, 4 larves libres.

Dans l'intestin, nous ne trouvons aucune larve.

Il est peu probable croyons-nous, que la mort de ce serpent puisse être attribuée aux larves de *Porocephalus*. Il est plus admissible que la capture de l'animal et les manifestations violentes nécessaires pour l'introduction forcée des larves ont provoqué les lésions hémorrhagiques et la mort.

De cette expérience nous pouvons déduire qu'il faut aux larves de *Porocephalus* introduites dans le tube digestif du *Causus rhombeatus*, bein peu de temps pour en sortir et parvenir dans les sacs pulmonaires. Entre le moment de l'introduction des larves, chez ce *Causus*, le 25-IX-08 et l'autopsie le 28-IX-08, il s'est écoulé *moins de 3 jours*.

Serpent No. 2. *Bitis arietans*, reçoit le 25-IX-08, de la même façon que le serpent No. 1 et du même singe, 9 larves de *Porocephalus* enkystées.

Le serpent avait été quelque peu malmené pendant l'introduction des larves, mais paraissait avoir bien supporté les manipulations. Le 30-IX-08 nous lui donnons en nourriture un rat qu'il tue et avale, mais ensuite le serpent reste très indolent. Le lendemain 1-X-08, l'animal meurt, à peu près exactement 6 jours après l'infestation.

A l'autopsie, nous trouvons dans le tissu cellulaire au niveau de la bifurcation des bronches et du premier sac pulmonaire, des suffusions hémorrhagiques et une congestion violente de ce sac pulmonaire.

A ce niveau, dans le tissu conjonctif en dehors du sac pulmonaire, nous trouvons 1 Porocephale libre, un autre parasite libre dans le tissu cellulaire un peu en arrière du pylore. Dans le sac pulmonaire antérieur, il y a 1 parasite libre; dans le sac pulmonaire postérieur près de l'estomac 4 Porocephales libres, qui n'ont pas produit la moindre irritation. Le 9e parasite n'a pas été retrouvé.

Quand à la mort du serpent, nous devons faire les mêmes remarques que pour le serpent No. 1 *Causus rhombeatus*.

Entre le moment de l'infestation et celui de l'autopsie, il s'est écoulé 6 jours. Les parasites retrouvés chez le serpent No. 2, étaient déjà manifestement plus grands que ceux du serpent No. 1.

Serpent No. 3. *Causus rhombeatus*, est infesté le 25-IX-08, de la même façon que les 2 serpents précédents, au moyen de 5 larves enkystées du singe.

Ce serpent reste bien portant et est tué au bout de 2 mois, le 25-XI-08.

Les 5 Porocephales sont retrouvés dans les sacs pulmonaires, attachées aux parois par leurs crochets.

Il résulte de ces expériences :—

1° L'infestation des petits serpents mis en expérience est bien une infestation *expérimentale*, et non une infestation naturelle pré-existante.

En effet, chez le serpent No. 2, nous avons retrouvé 8 parasites des 9 introduits. La place occupée par l'un des parasites, dans la partie terminale du gros intestin, près l'anus, indique que dans certains cas, une partie des larves peut être éliminée par cette voie.

Cela a été le cas sans doute pour la 9e des larves introduites. Chez le serpent No. 3,—5 larves ont été données, et 5 parasites ont été retrouvés dans le sac pulmonaire.

En outre la place occupée par certains Porocephales dans le tissu cellulaire chez les serpents 1 et 2, indique suffisamment, croyons-nous, que ces parasites étaient d'introduction récente.

2° La migration des larves introduites chez le serpent par voie stomacale, se fait rapidement: chez le serpent No. 1, en moins de 3 jours, des parasites sont parvenus dans le sac pulmonaire.

Ces nouveaux essais réussis d'infestation expérimentale de petits serpents par larves de *Porocephalus*, confirment ce que nous avons dit précédemment: *dans la nature les grands serpents s'infectent en avalant des animaux infestés de jeunes Porocephalus, pour lesquels ils constituent des hôtes définitifs.*

Nous avons recherché si un autre animal, par exemple, le singe, ne pouvait pas constituer un hôte définitif pour les *Porocephalus*, bien qu'après toutes nos observations, cette éventualité nous parût peu probable.

Le 1-X-08, nous donnes per os à un singe *Macacus*, 5 *Porocephalus* provenant du serpent No. 2. Les parasites mis dans une capsule en gélatine sont introduits profondément dans l'arrière gorge de façon à éviter toute lésion ou déchirure par les dents.

Le singe reste bien portant. Tué le 10-XI-08, nous ne trouvons pas de traces des parasites introduits.

Le singe ne peut donc constituer un hôte définitif pour les *Porocephalus*.

II. INFESTATIONS EXPÉRIMENTALES DE L'HÔTE INTERMÉDIAIRE

A. L'homme. L'une de nos malades, *Gwangwate* qui avait ingéré de l'eau avec des œufs de *Porocephalus* du serpent No. 2, était encore en vie au moment de la publication de notre seconde notice (voir ces *Annals*, Vol II, pages 320 et 321). Cette femme, arrivée à un stade incurable de la trypanose, avait été infectée par des œufs de *Porocephalus*, le 27-IV-08. Elle avait continué le traitement à l'atoxyl jusque peu de mois avant sa mort. Jamais cette femme n'a présenté le moindre symptôme morbide pouvant être

attribué à la presence de *Porocephalus* dans ses organes, ou même permettant de soupçonner l'existence de ces parasites dans son organisme. La femme est morte de trypanose, le 11-XI-09, c'est-à-dire, $18\frac{1}{2}$ mois après l'ingestion des œufs de *Porocephalus*.

Autopsie. Corps assez amaigri, pas de lésions cutanées.

Abdomen: pas d'exsudat dans la cavité péritonéale, pas de trace d'irritation. Nombreuses larves libres entre les anses, intestinales, dans les replis du mésentère, dans le petit bassin; elles sont mollement accrochées par leurs crochets. Nous recueillons en tout 73 larves libres.

D'autres larves sont encore enkystées dans le grand épiploon. Le foie renferme un nombre très considérable de larves enkystées, chaque incision faite dans l'organe, met à nu des larves enroulées et entourées de leur membrane kystique. Ni la rate ni les reins ne renferment de larves.

Cage Thoracique: pas d'exsudat dans les plèvres, pas de symptômes d'inflammation. Le poumon gauche renferme dans le lobe supérieur 2 larves enroulées et enkystées, le poumon droit, 1 seule larve.

Dans les *ganglions* lymphatiques du mésentère, nous retrouvons de rares larves assez petites, enkystées.

Pas de larves dans la paroi de l'intestin ni à l'extérieur de ce canal.

Les *dimensions* de ces larves sont relativement réduites: les plus grandes atteignent environ 22 mm. de longueur sur 2 mm. de largeur. Le plus grand nombre ne mesure que 15 à 16 mm. De cette analyse sommaire, deux points sont à retenir. D'abord les dimensions relativement réduites des larves, malgré qu'il se soit écoulé depuis le moment de l'infestation jusqu'à la mort, le temps considérable de $18\frac{1}{2}$ mois.

Nous devons en déduire que dans l'organisme humain, les larves de *Porocephalus*, se développent avec une lenteur extrême. Ensuite, l'absence de toute irritation, de toute réaction de la part des organes, constatée chez la femme Gwangwate comme chez les deux autres malades (ces *Annals*, Vol. II, pages 309-310), nous permet de conclure que les larves de *Porocephalus* ne provoquent guère d'irritation dans les corps humain.

B. Le Singe. Au moment de la publication de notre 2e note

(ces *Annals*, Vol. II, No. 4) le singe No. 4 (page 307) était encore en vie. Ce singe *Macacus* avait été infesté le 7-III-08 'avec des 'œufs de *Porocephalus* du serpent No. 1, gardés dans de la terre 'depuis le 7-XII-07, c'est-à-dire, depuis 3 mois. Les deux 'premiers mois la terre fut gardée humide, le troisième mois elle 'fut négligée, et resta plutôt sèche.'

Ce singe était resté constamment bien portant et très vif, lorsque le 5-II-1910, au matin, il est trouvé mort. Même la veille de la mort, nous n'avions pas remarqué chez le singe des symptômes de maladie.

Autopsie. Dans la cavité abdominale, nous trouvons de l'exsudat sanguinolent assez abondant, et une inflammation aigue du péritoine et de l'intestin. Nous y recueillons 100 larves vivantes et libres, réparties entre les anses intestinales, dans le petit bassin, ou accrochées à la paroi abdominale. Le grand épiploon renferme encore d'assez nombreuses larves enkystées.

Le foie présente une larve enroulée et enkystée à sa face antéro-supérieure et une seule larve petite, enkystée dans le parenchyme de l'organe.

Ni la rate, ni les reins, ni l'intestin, ne renferment de larves.

La cage thoracique est dans un état normal, les poumons ne renferment pas de larves.

A la face supérieure du diaphragme nous trouvons une seule larve enroulée et enkystée.

Les *dimensions* des larves libres recueillies dans la cavité abdominale sont très variables. La plus grande mesure 18 mm. en longueur, sur 2 mm. en largeur, la plus petite n'a que 8 mm. sur 1·5 mm.

Ce singe a succombé à une péritonite suraigue. Peut-elle être attribuée à la présence des larves *Porocephalus*?

Si l'on compare l'état macroscopique constaté chez nos autres singes infectés de *Porocephalus* soit naturellement soit expérimentalement, si l'on se rappelle l'absence complète de tout symptôme inflammatoire chez les 3 nègres infestés expérimentalement; l'on doit admettre avec nous qu'il est peu probable que la péritonite du singe No. 4 puisse être attribuée aux larves de *Porocephalus*.

Si elle pouvait avoir été provoquée par ces parasites, pourquoi

ne s'est-elle pas produite plus tôt chez ce singe infesté depuis près de deux ans?

Nous estimons que la péritonite du No. 4 peut être attribuée tout aussi bien à une cause fortuite, traumatique, par exemple. En effet, l'animal gardé à une chaine fixée autour de l'abdomen, a pu faire et faisait en réalité des mouvements violents. Une traction brusque, un choc, a pu produire un traumatisme abdominal, se terminant par la péritonite.

Quelle que soit la cause à laquelle l'on soit tenté d'attribuer la mort de l'animal, deux faits découlent de cette observation.

En premier lieu, c'est la *longue vitalité des œufs de Porocephalus*. Ce singe fut infesté comme nous l'avons dit plus haut avec des œufs de Porocephalus gardés pendant 3 mois dans de la terre, en somme dans de mauvaises conditions.

Ensuite nous ferons ressortir comme pour la femme Gwangwate, le développement extrêmement lent des larves de *Porocephalus* dans l'organisme de l'hôte intermédiaire-singe. Après 23 mois les plus grandes larves, rares, n'atteignaient que 18 mm., la moyenne 12 à 13 mm., les plus petites 8 mm. seulement.

Discussion Générale.

Des faits relatés dans cette notice comme dans les deux précédentes, nous pouvons déduire quelques faits intéressants pour l'histoire du *Porocephalus armillatus*.

Et tout d'abord, en ce qui concerne les hôtes intermédiaires, nous avons montré qu'en dehors de ceux signalés jusqu'à présent, homme, singe, chien, girafe, hyène, d'autres animaux encore, comme le chat, le rat, sont susceptibles d'infestation. Nous avons montré ensuite, par nos essais d'infestation expérimentale de l'homme du singe, du rat et du chat, que l'hôte intermédiaire s'infeste en avalant des œufs de *Porocephalus* adultes provenant des grands serpents.

Par l'infestation expérimentale du singe No. 4 moyen d'œufs gardés pendant 3 mois dans de la terre, en mauvaises conditions nous avons prouvé *le grande résistance des embryons*.

Enfin, de nos observations, nous croyons pouvoir conclure que

l'hôte intermédiaire naturel, du *Porocephalus armillatus*, est bien le singe. Nous avons signalé en effet (ces *Annals*, Vol. II, page 304), que sur 31 singes, nous en avions trouvé 9 infestés naturellement, soit 29%. Si l'on met en regard, d'un côté, la fréquence de l'infection *naturelle* chez le singe et sa rareté chez d'autres animaux ou l'homme, d'un autre côté, le fait que les grands serpents africains sont en effet mangeurs de singes, notre conclusion paraîtra logique. En ce qui concerne *l'hôte définitif*, nos observations n'ont fait que confirmer les connaissance acquises que les grands serpents africains *(Python sebae) (Bitis gabonica)* étaient les hôtes des formes adultes de *Porocephalus*. Chez le serpent, les parasites arrivent à développement complèt, ils s'y fécondent, ils y pondent leurs œufs.

Par nos essais d'infestation expérimentale de serpents plus petits *(Causus rhombeatus, Bitis arietans)*, nous avons prouvé que le serpent s'infecte par l'introduction par voie buccale de larves de *Porocephalus*.

Nos expériences ont prouvé ensuite qu'il faut aux larves bien peu de temps pour cheminer du tube digestif jusque dans les sacs pulmonaires. Chez le serpent No. 1, *Causus rhombeatus*, il a suffi de moins de trois jours.

Quand à la voie suivie par les larves de *Porocephalus* dans le corps du serpent, nous devons admettre qu'elles sont capables de *percer* la tunique stomacale, pour voyager dans le tissu cellulaire et pénétrer ensuite dans les sacs pulmonaires.

Nos observations ont mis en lumière la différence considérable dans la rapidité de développement des parasites chez l'hôte intermédiaire et l'hôte définitif.

Chez l'hôte intermédiaire ou accidentel (femme Gwangwate) ou naturel (par exemple singe No. 4), les larves, après un séjour respectivement de $18\frac{1}{2}$ mois et de 23 mois, avaient à peine atteint 16 mm. et 22 mm. pour les formes les plus avancées. Par contre, chez l'hôte définitif, il a suffi d'un séjour de peu de jours, pour provoquer un développement marqué, appréciable à première vue.

Enfin, nos expériences et nos observations ont prouvé que les larves ou jeunes *Porocephalus* chez l'hôte intermédiaire soit naturel, ne provoquent guère de désordres. Parmi tous nos cas

d'infestation expérimentale d'hôtes intermédiaires, seul un singe (No. 4) présenta, après 23 mois d'infection, des symptômes macroscopiques d'inflammation. Nous avons dit plus haut que nous ne pouvions pas sûrement les attribuer à la présence des parasites.

De même nous devons remettre en doute l'explication que nous avons donnée de la mort du soldat, chez lequel nous avons constaté en 1907, un cas d'infestation naturelle par larve de *Porocephalus* (ces *Annals*, Vol. I, page 499).

Nous avions dit alors que la larve devait avoir été enkystée dans la rate, qu'elle avait à un moment donné dû rompre le kyste et provoquer des lésions dans la rate, qu'elle était sortie ensuite de l'organe pour se loger dans la cavité péritonéale. Bref, nous avions cru alors à une fente purulente de la rate, suivie de péritonite, maladie dont la cause primordiale aurait été la larve de *Porocephalus*.

Après toutes nos observations et nos expériences d'infestation cette explication ne peut être maintenue. Ni chez l'homme, ni chez l'animal infesté expérimentalement, nous n'avons trouvé de larves à l'intérieur de la rate, alors que d'autres organes abdominaux, notamment le foie en contenaient.

L'absence de toute irritation et inflammation chez les singes infestés naturellement, et chez l'homme et les animaux infestés expérimentalement (à part le seul singe No. 4), prouve à suffisance que la mort du soldat doit être attribué à une autre cause qu'à l'action de la larve de *Porocephalus*. Cet homme doit avoir eu une affection de la rate ayant déterminé la fente purulente de cet organe, et cette lésion s'est compliquée d'une péritonite aigue.

CONCLUSIONS

L'hôte définitif de *Porocephalus armillatus* sont les grands serpents, *Python sebae*, *Bitis gabonica*.

L'hôte intermédiaire naturel est le singe.

L'hôte intermédiaire accidentel peut être tout autre animal ou même l'homme.

L'hôte intermédiaire s'infecte en avalant des œufs de

Porocephalus éliminés par les grands serpents, les œufs renfermant un embryon offrent une grande résistance.

L'hôte définitif s'infeste en avalant un hôte intermédiaire naturel infesté.

Chez l'hôte intermédiaire, accidentel ou naturel, les larves de *Porocephalus* ont un développement très lent—le développement chez l'hôte définitif est rapide

Les *Porocephalus* introduits dans l'estomac de l'hôte définitif percent la tunique stomacale et arrivent dans les sacs pulmonaires par migration à travers le tissu cellulaire.

Chez l'hôte intermédiaire, les larves de *Porocephalus* ne provoquent de lésions anatomiques ou inflammatoires que dans des circonstances exceptionnelles.

ON THE ABSENCE OF A VESICANT IN THE ETHER EXTRACT OBTAINABLE FROM MOSQUITOS

BY

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The bites of mosquitos are well known to cause a more or less troublesome degree of irritation of the skin, which commences at the end of a few hours, and lasts from one to three days or more. The skin in the immediate neighbourhood of the bite becomes reddened, itchy and slightly swollen. If rubbed a wheal is generally readily produced. The degree of irritation varies with different individuals. Those who have lived long in mosquito-infested districts not unfrequently describe themselves as having become quite or nearly immune to mosquito bites.

The cause of this irritative effect of the bite of the mosquito is presumably to be found in the fluid injected by it into the skin when sucking blood.* The nature of the effective constituent of this fluid has not yet been determined and, owing to the small size of the mosquito, investigation is attended with difficulty.

The experiments which form the subject of this paper were made with a view of ascertaining if any substance possessing irritating properties when applied externally to human skin was present in the extract obtainable from mosquitos with the aid of ether. It is well known that in the Spanish Fly (*Lytta vesicatoria*) a strong vesicant, cantharidin, is present in amount, equal, it may be, to as much as 2 per cent. of the dried insect. Although the present investigation failed to reveal the existence of any irritant in mosquitos, similar to that present in *Lytta vesicatoria*, it

* F. Schaudinn (Generations und Wirtswechsel bei Trypanosoma und Spirochaete. Arb. aus dem Kais. Gesundheitsamte, 1904. Bd. 20, S. 419), finds that the contents of the oesophagus of the mosquito are effective in causing irritation of the skin probably by the agency of the fungi they contain. The salivary glands are not irritant.

may be of interest to any who contemplate working on similar lines to give the method employed.

The procedure followed was similar to that adopted in the extract of cantharidin from Spanish Fly. About 500 mosquitos, consisting of various species of Culicines and Anophelines (chiefly the former) were collected in Nyasaland (Upper Shire River). The weight of these, after drying at 110° C., was 0·478 gr. The dried mass thus obtained was ground in an agate mortar, and mixed with one-third of its weight of magnesia. Water was then added, and the mixture evaporated to dryness in a porcelain capsule on a water bath. Dilute sulphuric acid was then added until an alkaline reaction was no longer obtainable, after which the water present was again driven off on a water bath. The dried mass thus obtained was next extracted with ether in a Soxhlet apparatus. From the extract thus obtained ether was removed by distillation, and the residue kept at 100° C. until all water was driven off. The residue was then again dissolved in a small amount of ether, and applied drop by drop to an area of human skin (flexor surface of the wrist) about 15 mm. in diameter. A small amount of solid material, not taken up by ether, was further extracted with chloroform, which was then allowed to evaporate on the same area of skin.

No irritant effect was, however, produced, the skin remaining free from redness or itching. The area to which the ether extract had been applied was kept undisturbed for fourteen hours without any change manifesting itself.

It would appear, therefore, that the irritant action of mosquito bites cannot be attributed to the existence in these insects of any substance possessing a vesicant action.

FACTORS IN THE TRANSMISSION AND PREVENTION OF MALARIA IN THE PANAMA CANAL ZONE

BY
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INTRODUCTION

In every malarial region it is important that the species of mosquitos common to that region should be recognised, their breeding habits should be studied, and a determination made of the species of Anophelines, hospitable to malaria, and those transmitting it. The English observers, Stephens and Christophers, noticed that certain species of Anophelines were natural transmitters of malarial fever, while others were rarely, if ever, found infected naturally, although it was possible to infect them under laboratory conditions. We know that the breeding habits of Anophelines vary, too, considerably, and it may be said that there is as much selection of breeding places by Anophelines as there is selection of feeding grounds by fish. Trout, salmon, and bull-heads have their analogues among Anopheline larvae; some of the latter requiring fresh aerated water, or water containing much green algae. Others are found in tree-holes and recesses of epiphytic tree plants, such as Bromelias, where they prey upon other species, while others preferring fresh aerated water are so adaptable that they will flourish in sewage streams, or in brackish water containing half its volume of sea water.

Some species require an abundance of sunlight, while other sylvan species prefer shady pools in which chlorophyll-bearing algae are relatively absent. The Anophelines insusceptible to malaria may be more limited in their choice of breeding places, so that in the work of malarial mosquito destruction the latter may be

disregarded, and attention given wholly to the breeding places of those species responsible for the transmission of malarial fever.

With regard to man as a host, it is necessary to have some knowledge of the limits of his infectiousness, i.e., the number of sexual forms of the malarial parasite necessary to infect susceptible mosquitos.

Besides the question of hospitable species of Anophelines, there are other matters of much importance, such as latent malaria; the effect of quinine on the parasites in man; the value of various larvacides; algacides; agents destructive to ditch grass, and a knowledge of the quality of wire screening, and the size of the mesh necessary to keep out mosquitos.

OUTLINE OF THE SUBJECTS CONSIDERED:

Anophelines of this region.
Collection of larvae.
Breeding out mosquitos and methods of feeding.
Biting—infecting experiments.
Estimation of gametes.
Care of mosquitos after feeding.
Method of examining for zygotes and sporozoites.
Description of the malarial parasite in the mosquito.
Table of infecting experiments.
Notes and conclusions from table of infecting experiments.
Limit of infectiousness of man.
Notes on the bionomics of Anophelines.
Effect of salt or sea water on larvae.
Experiments with larvacides.
Experiments with agents destructive to vegetation, grass and algae.
Experiments with screening of various mesh.
Relative value of wire screening of various composition, based on practical tests and chemical analyses.
Note on the value of the practice of killing Anophelines found in quarters and barracks.
Effect of quinine upon the parasite in mosquito and man.

The following is a list of Anophelines of the Canal Zone: —

Cellia argyrotarsis, R.D.
Cellia tarsimaculata, Goeldi
Cellia(?) gorgasi, D.K.
Cellia albimana, Wied.
Anopheles (?) cruzii, D.K.
Anopheles (?) apicimacula, D.K.
Anopheles (?) punctimaculata, D.K.
Arribalzagia (?) malefactor, D.K.
Anopheles (?) eiseni, Coquill
Anopheles francisanus, McCrack
Anopheles pseudopunctipennis, Theob.

The above eleven species of Anophelines have been collected in the Canal Zone during the past five years. They are not taken, nor do they exist in their breeding places, in anything like equal numbers. For example: Only one specimen of *Ce. (?) gorgasi* has been found. Of the eleven species, the commonest ones are *Ce. albimana*, *A. pseudopunctipennis* and *Arr. (?) malefactor*, but this again must be qualified by stating that the predominance of a species varies from season to season and from place to place. In certain villages, upon going through the barracks only *Ce. albimana* will be found, while in other villages, from five to ten per cent. of the mosquitos will be *A. pseudopunctipennis*, and at Ancon during October, 1908, 27 per cent. were *A. malefactor* and 72 per cent. *Ce. albimana*. Mr. A. Busck, of the Bureau of Entomology, United States Department of Agriculture, who collected and made observations on Zone mosquitos during 1907, gave it as his opinion that *A. pseudopunctipennis* was the commonest Anopheline during the period of his stay.

The necessities of the canal operations in excavating and filling, change the topography of districts and localities so as sometimes to convert salt marshes into fresh water ponds, or to make tracts of land containing few Anophelines, into a vast swamp in which they luxuriate. On the other hand, swamps and breeding places may be drained or filled in the work of excavation. These factors, among others, influence the number and variety of species in a locality.

The commoner Anophelines of the Canal Zone may be divided into three groups: —

(A) The white hind-footed group comprising:

Ce. argyrotarsis,
Ce. albimana,
Ce. tarsimaculata.*

(B) The leg uniformly coloured group comprising:

A. pseudopunctipennis,
A. franciscanus.

(C) The spotted leg group comprising:

Arr. (?) malefactor,
A. (?) apicimacula.

These groups present well-marked differences in the markings of adults, in the breeding habits and anatomical characters of the larvae, and, as will be shown, they possess varying susceptibilities to malaria.

The following are descriptions of the species of Anophelines of the Canal Zone:—

Cellia argyrotarsis

Thorax with mesonotum bluish-grey, with three more or less longitudinal lines and with pale scales over the mesonotum, and sometimes traces of two dark lateral spots. The abodmen dark, dusky-brown, with a few creamy scales. Legs covered with dark scales, with some of the tarsi apically white banded; last three joints of hind legs pure white, and also apex of first; costa dark with two distinct and several smaller pale spots.

♀ Head black, with white upright spatulate scales in front, black behind and at the sides, a tuft of white hairs projecting forwards between the eyes. Eyes black; antennae dark, with pale silky pubescence and brown hair; basal joint dark, a few patches of white scales on the first few basal joints; palpi covered with long black scales, especially towards the base; apex pure white, and there are also two narrow white rings on the apical ends of the joints; ventrally, the penultimate joint has a number of yellowish-white scales, which sometimes seem to form almost a ring; proboscis clothed with short dark scales.

Thorax with a bluish-grey sheen, with three more or less distinct longitudinal lines, the middle one most distinct, and of a purplish hue, with pale scales scattered over the mesonotum; scutellum dark towards the middle; mesonotum deep brown; pleurae dark, with here and there frosty tomentum (there are traces of two dark lateral spots on the mesonotum, which are clearly seen in the St. Lucia specimen).

Abdomen dusky purplish-brown, clothed with creamy yellow scales, especially in the middle region of the segments; the segments have lateral tufts of grey scales on the posterior borders, projecting from the sides; hairs long, deep bright brown; viewed with a pocket-lens the abdomen is almost black in ground colour; in other specimens dull yellowish reflections may be seen.

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* According to Theobald (Monograph of Culicidae, Vol. V, p. 69) identical with *albimana*.—ED.

Legs yellowish, covered with dark brown scales; first two tarsi of the forelegs apically white, last two joints dark brown, four midtarsi also with small pale apical bands; mid metatarsi and first two tarsal joints with minute apical yellow bands, last two indistinctly banded; in the hind legs the last three joints are pure snow white, and also the apex of the first; ungues very dark.

Wings with the costa dark, with four distinct and several smaller white patches; there are also numerous patches of dark scales, which vary to some extent, over the wing areas; in the ♀, from which this description is taken, the fourth long vein is covered with pale dusky scales, whilst in a ♀ from St. Lucia, it is creamy white; halteres with pale stem and fuscous knob. Length, 4 to 5 mm.

♂ Palpi dark brown, with scattered white scales, especially on the last swollen joints; hair-tuft pale; there is a pale ring at apex of the apical and base of the penultimate joint; antennae brown, with brown plumes; proboscis brown and narrow. The white scales on the head extend nearly over the neck; scales on the thorax white; the larger ungues of the fore-feet biserrated. Length, 4 to 5 mm.

During the period in which these experiments were being conducted I received very few specimens of this species, the sources being Miraflores, Ancon, Culebra, Paraiso and Corozal. Two specimens of *Ce. argyrotarsis* bit a patient having one crescent to 200 leucocytes and neither became infected. The patient was possibly an unfavourable case, and the experiment was not controlled by biting susceptible *Ce. albimana* at the same time. On December 2nd, from some Anophelines collected in labour cars at Corozal, one specimen of *Ce. argyrotarsis* was found containing a malarial zygote, 29μ in diameter, with fine discrete pigment.

Cellia tarsimaculata

This mosquito resembles *Ce. albimana* very closely, except for the different arrangement of the white bands on the palpi. This mosquito was found to transmit malaria.

Cellia albimana

This form resembles the type in all respects except that the last tarsal joint in the hind-legs has a very distinct and persistent deep black basal band. The thorax is rather browner in some specimens, and there are only two white bands to the ♀ palpi. The forelegs have dark-scaled femora, pale underneath, with a small white knee spot, the tibiae dusky-scaled and also the metatarsus above, pale below, apex white; the first two tarsi have yellow apical bands, the third dark, and the last clay coloured; mid legs with a large white spot near the apex of the femora; mid tarsi not definitely banded, but with a faint pale band sometimes at the apex of the metatarsus; the hind legs are dark brown, with the second, third, and apex of the first tarsal joints pure white, the last joint white, with a distinct black basal band; ungues as in the type. Wings much as in the type, but the pale scales are more yellow in colour. Length, ♂ 3·5 to 4·5 mm.; ♀ 4 to 4·5 mm.

This was the commonest species of Anopheline received as adults or larvae during the period embraced by this work, and was found to transmit both malignant tertian and simple tertian malaria.

Cellia (?) gorgasi

Palpi as long as the proboscis, mostly black scaled, the terminal and penultimate joints light scaled, except at the bases and apices; mesothorax grey with fine brown scales, a black spot in front of the scutellum, a pair of sublateral black spots medially; wings with the veins scaled in black and white, two very

large black patches on the costa and a smaller one towards the base and a smaller one at the apex as in *Ce. albimana,* Weid. The rest of the wing is too much denuded to describe. Abdomen with groups of outstanding scales laterally at the apices of the segments, the dorsum clothed with yellow scales on a dark brown ground, the lateral tufts black. Legs mostly black-scaled, hind legs with the apical half of the second, the third, and the base of the fourth joints white-scaled, the remainder of the fourth and basal half of the fifth segments black, the third joint with a large black patch on the under side which reaches from near the base to beyond the middle. Length, 3·5 mm.

A single adult female of this species was collected by Mr. A. H. Jennings.

Anopheles pseudopunctipennis

Wings much as in *A. punctipennis,* Say, but the fringe with yellow spots. Legs, long, unbanded, brown, pale at the base. Fore ungues of ♂ unequal, mid and hind equal and simple.

♀ Antennae brown, basal joint testaceous, base of the second joint pale, and also a small pale band at the base of all the following joints: proboscis dark brown; labella yellowish; palpi dark brown, densely scaled at base; apex yellow, and also two narrow yellow bands below, slightly hairy, hairs black, except at the apex where they are yellow; clypeus dark brown. Thorax yellowish-brown (denuded), with a dark patch on each side of the mesonotum behind; metanotum deep brown; pleurae yellowish brown, with darker brown patches. Abdomen brown, the segment paler at the base, hairy. Legs deep brown; coxae, trochanters and base of femora pallid; knee spot pale; ungues equal and simple. Halteres with pale stem and fuscous knob.

Wings with two yellowish white spots on the upper costal border, rest of the edge black, rather densely scaled; first submarginal longer and narrower than the second posterior cell, its stem nearly as long as the cell; mid cross-vein a little nearer the base of the wing than the supernumerary cross-vein; posterior cross-vein still nearer the base of the wing; scales of the wings disposed as follows:—First long vein with three distinct large white spots, one at the base, one underneath a large costal spot, and one between; second long vein with a dark patch near its base, all the lower branch of the fork-cell dark, and most of the upper; third long vein mostly yellowish-white, with two black patches, one towards the base, and the other towards the tip; fourth long vein mostly pale, with two small black patches, branches of the fork-cell all dark scaled; fifth long vein with a black spot near the base, rest mostly yellow, upper branch of the fork mostly dark, a small yellow spot at the apex and another towards its base, lower branch mostly yellowish, with a black apical spot; sixth vein with the basal half creamy, the apical half dark, except a small yellow patch where it joins the wing border; fringe brown, with yellow spot at the junction of each vein. Length, 5 mm.

♂ Last two joints of the palpi swollen and clavate, pale; basal joints dark brown, densely scaled with deep brown scales, with a narrow pale band not quite as long as the thin proboscis, which is brown with yellow labella; antennae grey, with narrow brown bands and flaxen brown hairs, the apical joint about half the length of the penultimate joint; basal lobe of the genitalia simple, claspers long and thin; fore ungues unequal, the larger one uniserrated, the smaller minute and simple; mid and hind ungues small, equal and simple. Wings much as in the ♀ but the fork-cells shorter. Length, 5 mm., with proboscis 7·5 mm. Habitat, Grenada (Dr. Hayton, per Dr. Daniel). Time of capture, February.

Observations—Very like *A. punctipennis,* Say, but can at once be told by the wing fringe being spotted at the apex of each nerve, and by the marking of the sixth long vein. The description is drawn up from two specimens in balsam, so

that the scale structure is not evident. It is so very distinct, however, that it can easily be identified by the characters given below. I succeeded in infecting four specimens of this species.

Mr. August Busck found this species to be the commonest and most widely distributed one in the Zone during the season in which his collections were made, April-July, 1907.

Anopheles franciscanus

Male: Head dark brown, with short, dark, erect scales towards nape, emarginate and slightly forked, vertex and anterior part of occiput, with short, light brown scales not forked, a tuft of light brown hairs projecting forwards between the eyes, a row of similar hairs projecting forwards encircling the eyes posteriorly; eyes deep purplish brown; antennae about two-thirds length of palpi, yellowish-brown hairs, basal joint dark brown; palpi equalling proboscis in length with emarginate scales from base to tip on under and outer surfaces, those upon outer surface dark, upon under surface light, long light hairs covering distal third, becoming short and stout at the apex, a slightly banded appearance at base of three distal segments; two distal joints spathulate, proboscis scaled except labella, labella covered with medium stout setae, a few light hairs at apex.

Thorax: Prothoracic lobes dark; mesothorax dark brown at the sides, with scattered light hairs, a broad light-brown patch in the middle; within this light area a median line and obscure lateral lines; scutellum light with single horizontal row of hairs; metanotum dark without hair; halteres dark, covered with thick pubescence and emarginate scales; stalks light without scales.

Abdomen, basal area of each segment light, covered sparingly with long, light hairs; two stiff hairs on posterior margin of distal segment, stout hairs on margin of genital lobes.

Legs, coxae and trochanter light; trochanters, femora, tibiae, and tarsi covered with short, dark, emarginate scales and setae; ungues of front legs very unequal, the larger one with a large median tooth and a smaller basal lobe; middle ungues covered, with blunt basal lobes; posterior ungues equal, simple; posterior metatarsus slightly longer than tibia.

Wings with dark costa, with two distinct, nearly equal, yellow spots—one at distal end of subcostal vein, one at and involving distal end of first long vein; fringe dark, with a yellow spot at the end of each vein except at the end of the sixth; the first spot carried on to the first long vein, the apical spot carried past long vein on to the upper branch of the second long vein; the second long vein dark except for a few basal light scales; third long vein yellow in the middle, dark at the base and apex; light area at base of third long vein, carried over the fourth on to the upper branch of the fifth, with a few light scales at base; main branch of fifth long vein light, except at base and apex; distal half of sixth long vein dark, except at apex, basal half light; sub-costal with a light spot carried to the first long vein (in one specimen the light spot on sub-costal missing); third long vein prolonged slightly into the basal cell; first sub-marginal longer and slightly narrower than second posterior cell, stem twice the length of the cell; stem of second posterior cell prolonged to base of wing; supernumerary cross-vein adjacent to, or but very shortly removed from mid cross-vein and equal to it in length when removed nearer to apex of wing; posterior cross-vein a little longer than mid cross-vein and varying in distance from it, from one-half to almost twice its own length; third long vein prolonged slightly into the basal cell, darkest scales on costal, sub-costal and first long veins.

Palpi of the female equalling proboscis in length, light area at base of three distal segments, giving a banded appearance, clothed with scales, short hairs and

setae, as in male, distal joints not spathulate; legs with the ungues equal, otherwise with the male.

No specimens of this species were infected, but as they are so close to *A. pseudopunctipennis* it might have been possible to infect a few if a large number had been used.

Arribalzagia (?) malefactor

♀ Palpi long, clothed with brown scales and black outstanding ones, which are grouped more or less in tufts, heaviest on the basal portion, a slight sprinkling of lighter scales among the brown ones, particularly at the bases of the dark tufts; occiput black scaled, the eyes margined with white above, and where they join it a tuft of white hairs; mesonotum grey with feddish and bluish tinge and small dark freckles tending to form longitudinal rows, sparsely distributed narrow yellowish scales, a spot at the base extending over the middle of the scutellum and two small sub-lateral back spots medially, all three of these show a lighter margin; abdomen slender, grey, with lateral tufts of outstanding black scales at the apices of the segments; legs with the femora and tibiae black, freckled with white; on the hind tibiae yellow scales predominate; tarsi black, ringed with yellowish-white; on the hind legs the first tarsal joint is dark at the base, light at the apex and has six white rings of different lengths, second joint narrowly white at base, broadly so at apex, with a moderately broad white ring near the middle and another narrower one between it and the base, third and fourth joints white ringed at base and apex with a broad central white ring, apical segment entirely whitish scaled; wing spotted, black and white, a large black patch margined with white on the costa near the middle, more basally a smaller costal patch and towards the apex another large one, all margined with white, scaling of the veins in patches of black and white scales, the third vein with a small black spot at the base, the sixth vein with many black dots and dashes. Length, 4·5 mm.

♂ Palpi with the apical portion clubbed, clothed with yellow scales with golden lustre, a narrow dark ring at the middle of the club, the shaft ringed with dull ochreous at the apex and at the constriction and broadly marked with the same colour on the apical portion; antennae pale brown and ferruginous with silky lustre. Length, 4·5 mm.

This large and beautiful Anopheline was received in good numbers from Miraflores and Ancon. Its name, however, appears to be a misnomer, for it could not be infected with malaria under the most favourable conditions.

Anopheles (?) apicimacula

As in *A. strigimacula*, D. and K., but with a distinct black costal apical spot on wing.

Anopheles (?) strigimacula

Proboscis black; palpi as long as the proboscis, black, a few whitish scales at the bases of the last two and middle of the long joint. Occiput black, clothed with erect black scales. a group of white ones in the centre of the vertex, a tuft of pale hairs at the vertex.

Mesonotum narrow, elongate, greyish, pruinose, a black spot below the lateral angle and one on the ant-scutellar space; vestiture of fine pale hairs arising from small black tubercles. Scutellum collar-like, greyish, with a black spot in the middle, clothed with pale bristles. Pleurae and coxae blackish with fine hairs, the coxae with patches of white scales.

Abdomen with the tip truncate, brownish black, clothed with numerous fine pale hairs; a row of lateral segmental posterior tufts of black spatulate outstanding scales; beneath with tufts of black scales and with scattered white ones.

Wings hyaline, the petiole of the second marginal cell as long as its cell; basal cross-vein distant about its own length from the anterior cross-vein; scales of the veins ovate, white on the costa and first vein, pale yellow on the others, with black scales and spots as follows :—

Three costal spots, the first small, involving two veins, the others large, involving three veins, the membrane beneath infuscated; no apical spot; costa and first vein with two or three little black spots between each of the large ones, the outer spot involving the base of the fork of the second vein; each fork with two little spots beyond; third vein with two spots at the base and two at the tip; fourth vein with a spot at the base, a large one involving the base of the fork, three on the upper branch and two on the lower; fifth vein with some black scales at the base, five spots on the upper fork, two on the lower; sixth vein with some irregular black scales toward the base, a spot in the middle and one at the tip.

Legs long and slender, black, speckled with white. Femora with about eight spots; tibiae with about fourteen, being about as many black scales as white ones; hind tarsi with ten spots on the first joint, second, third and fourth joints white at the base and tip, with a ring beyond the middle; fifth joint all white. Front tarsi with narrow white rings at bases and apices of the joints, the last entirely pale; mid tarsi not distinctly ringed. Claw simple. Length about 5 mm.; of the wing, 4 mm.

Anopheles (?) punctimaculata

As in *A. strigimacula,* D. and K., but the last vein with a row of black dots.

Only one specimen of this species is known. It was taken at Colon (W. M. Black, collector).

Anopheles (?) cruzii

This species was examined only in the larval state, through the kindness of Mr. A. H. Jennings. No adult specimens were obtained and none ever received from or taken from quarters. This is significant on account of the peculiar tree-living habits of this species, and the probably groundless fear that it might be a malaria carrier.

Anopheles (?) eiseni

Near *A. maculipennis*, but with a patch of whitish scales on the first vein before its middle and another at its apex, also the apical fourth of the hind tibiae is yellowish-white. Halteres black, the stem whitish; coxae and a vitta on lower part of pleura, yellow, femora yellowish-brown, apical fourth of hind tibiae yellowish-white; antennae of male whitish, the first joint, last two, and fascia on each of the others, brown; scales of palpi black, those of apex and two bands in the female, three in the male, white; scales of occiput black, those in the middle of upper part white; mesonotum greyish pruinose, marked towards each side with a velvet black vitta; scales of abdomen black, the hairs yellowish, scales of femora and tibiae mixed black and whitish, those on the apical whitish portion of hind tibiae white, those on the tarsi black; tarsal claws on female simple; wings hyaline, the veins and scales brown, a dense patch of black ones at base of second vein, a larger one on the cross-veins and a smaller one at bases of first sub-marginal and

of second posterior cell, a small patch of yellowish-white scales on first vein before its middle and another at its apex, the latter spot encroaching upon the costal vein. Length, 3·5 mm.

It would have been of considerable interest to determine the susceptibility of *A eiseni* and *A. cruzii* to malaria on account of their peculiar tree-living habits, but it was almost impossible to obtain larvae of those species; and among hundreds of Anophelines caught within quarters and barracks which were examined and dissected, no specimens of this species were ever seen. It is extremely unlikely that they play any part whatever in the transmission of malarial fever in the Canal Zone at this time. The larvae of these species proved extremely interesting in comparison with the commoner species, such as *Ce. albimana*, *A. pseudopunctipennis* and *Arr. (?) malefactor*, the anatomical characters of the former indicating definitely a very marked alteration in habits.

COLLECTION OF LARVAE

In order that a large number of adults could be kept on hand from day to day, it was arranged that sanitary inspectors at the various districts along the Panama Railroad should send bottles containing larvae and pupae to the laboratory daily. Special collections of larvae were also made and excursions to breeding places made from time to time. Upon receiving them at the laboratory, larvae were transferred to a glass moist jar partly filled with fresh water. Predaceous larvae, such as dragon-fly larvae were removed or killed and the Anopheline larvae transferred to feeding tanks containing algae and organic débris. These glass breeding tanks were placed on a table in front of a window having an eastern exposure so that they got direct sunlight for a few hours in the morning. The water in the breeding tanks was kept fresh and free from fouling by passing a jet of air through it with a Pacquelin cautery bulb, having a heavy glass perforated tip. This proved to be a very important addition to the technique of breeding out larvae. For shade and shelter a few *Lemma* plants were placed in the tank.

Several writers, collectors, and malarial investigators have mentioned the difficulties attendant upon the breeding out of Anopheles mosquitos from ova or very young larvae. Others have not mentioned the difficulties encountered or have not described the means used to obviate them. Banks, in the *Philippine Journal of Science*, Vol. II, No. 6, December, 1907, states, 'In laboratory 'breeding experiments the plants in the water begin to die within

'three to five days, while the larvae appeared to feed in a desultory 'manner. The time for their natural transformation to the pupae 'comes and goes and they still remain as larvae. The foulness of 'the water, due to organic decomposition, appears not to affect 'them, but on the other hand the lack of proper food seems to cause 'them to remain in an indefinite larval state until after three weeks 'or more they gradually begin to die.'

A very simple and satisfactory method for keeping the breeding tanks fresh and clean and free from decomposition was devised. The principle involved is that of aeration—preventing the development of an anaerobic condition in the water of the breeding tank by passing a fine jet of air through the water of the tank once or twice a day. It is well known, of course, that if natural water from streams, pools or ditches be placed in a glass tank, exposed to diffuse sunlight and undisturbed, after a few days the chlorophyll-bearing plants begin to disappear, a pellicle forms on the surface of the water, which contains bacteria, spirilla, flagellated protozoa, amoebae, etc. Beneath this surface film decomposition goes on rapidly, chlorophyll-bearing forms are destroyed or become encysted, and the various insect larvae and water-bugs die. The bacteria in the deeper portions of the water are largely anaerobes, and are associated with the putrefactive decomposition of the vegetable matter and animal matter in the tank. Banks describes exactly the effect on mosquito larvae of this putrefying vegetable matter. The odour arising from the tanks suggested the necessity of aeration to prevent the growth of the anaerobic bacteria. Acting on this suggestion an air jet was devised by attaching a thick glass rod, having a fine capillary central canal to the double bulb of the Pacquelin cautery apparatus and the breeding-out jars aerated for a minute or two morning and evening. The results were highly satisfactory, for the tanks were by this means kept clean and wholesome. The algae remained green and vigorous, the larvae active and developed rapidly into pupae, the water remained quite clear, in fact, with its floor of sand and clay and a few sprigs of green aquatic plant, such as *Lemna*, looked as tempting to drink as spring water. The temperature of the water in the tanks ranged between 72° and 84°.

BREEDING OUT—METHODS OF FEEDING

Pupae were collected in the morning and evening and placed in breeding-out tubes half filled with water and plugged with cotton. Each morning the newly emerged mosquitos were transferred to biting-jars. These biting-jars were modified from those described by Stephens and Christophers. After trying pickle jars and malted milk jars with much impatience, jars made of lantern chimneys were used. These were covered on both ends with crinoline gauze, fastened with a string or a strong rubber band. Inside the jar was placed a circular ring platform of stiff paper, which many of the mosquitos used as a resting place. About twenty mosquitos would be placed in a jar over a small Stender dish* containing water on a Petri dish cover with a raisin or a piece of date for food. The jar would then be placed on a shelf in a dimly-lighted place, protected from ants by kerosene cups. Adult mosquitos may be kept alive for several days in such jars if they are fed with dates or raisins and a few drops of water, but if fed daily they will not bite and suck blood with alacrity. Anophelines do much better if they have one or two preliminary blood-meals before being fed with dates, raisins or bananas. In several experiments it appeared that in the mid-gut of mosquitos gorged with stale banana and the associated bacteria and yeasts, the fermentative acid contents either destroyed the flagella of gametes, or in some other way prevented the development of zygotes in the mid-gut of susceptible mosquitos. In one experiment a patient whose blood contained almost as many crescents as leucocytes was bitten by four varieties of mosquitos; three of the four being susceptible species, yet none became infected. This failure might be attributed to the feeding on bananas.

Until the mosquitos were used for biting they were fed with dates, raisins and bananas, whenever they were to be kept for biting and dissections, it was found that those fed on dates and raisins gave much better dissections, while the mid-guts of banana-fed mosquitos frequently contained many yeasts and bacilli which caused the death of some of the mosquitos, apparently as a result of fermentation, and frequently yielded partly softened, tender and

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* A ' Stender-dish ' is one with a grooved lid fitting the rim of the dish.—EDS.

disintegrated intestinal tracts, which were removed intact with difficulty, and were occasionally lost. Bananas should never be used as food when dates or raisins can be obtained.

Mosquitos were kept in these biting-jars until a suitable case of malaria presented. The blood of patients selected to be bitten contained gametes or sexual forms of the malarial parasite in their circulating blood in numbers sufficient to infect susceptible anophelines. The method of determining this point will be considered later.

BITING AND INFECTING EXPERIMENTS

The earlier biting experiments were conducted at about 8 o'clock in the evening. After selecting a patient the jar of mosquitos was placed on the patient's forearm and covered with a heavy towel to prevent the disconcerting effect of light. Females several days old, that had been fed exclusively on dates or raisins, would generally bite greedily, and would feed on successive or alternate nights if given an opportunity. Most females would bite twenty-four or more hours after emerging, but before that period they would generally make no attempt to do so. When a jar is placed over a patient's forearm the mosquitos that are going to bite will almost always do so within a few seconds. If they show no inclination to visit the arm, a few gentle puffs into the opposite end of the jar, through the crinolin gauze, will make them change their position and frequently take up one on the patient's forearm. Another method that was used successfully was to have the patient's bed in the dark. If the jar were then gently tapped two or three times and aimed at a light down the ward some distance away the mosquitos would always take up a position on the gauze facing the light. The patient's forearm could then be carefully interposed and placed on the gauze, when the mosquitos would frequently take the hint and feed. Strong lights are powerfully attractive to female Anophelines and interfere considerably with successful bitings unless the jars are darkened by means of a thick cloth, the patients taken into a dark room, or the mosquitos very hungry.

Later, it was more convenient to conduct the biting about

4 o'clock in the afternoon, and on these occasions it was generally necessary to cover the jars well with a thick towel.

Two classes of patients were used: Spanish labourers and West Indian negroes, the former rarely objected to being bitten, the latter occasionally fretted a little. Both classes of patients were rather obtuse mentally and occasionally would not feel the bite, or would not be able to tell correctly the number of them. One reason for this is to be found in the limits of the number of separate points of pain appreciable within a radius of three inches—the biting area of the jar.

The Spanish patients' hands occasionally reeked with the odour of cigarette smoke, and a few of the West Indians had used citronella to prevent sand-flies from biting, but in neither instance did this interfere with the feeding of mosquitos. The hungry females, if undisturbed, will gorge themselves with blood and not infrequently will expel a drop or two of bloody fluid per anum. This not only occurs at the first feeding but has been observed at subsequent feedings.

After as many of the females as will bite have done so, the jars are taken to the laboratory, one of the gauze covers carefully removed and at the same time replaced by a card which is slipped out when the jar is placed on a Petri dish. The Petri dish contains a small Stender dish with a few drops of water and a split raisin or two. The jar is then placed on an ant-proof shelf in a dimly-lighted place, and under these conditions, with fresh food and water, Anophelines may be kept alive for two or three weeks. If one has fed mosquitos late in the evening and does not care to transfer or uncover them by artificial light, the gauze may be moistened with a few drops of tap water and the jars placed on the shelf until morning.

At the time of biting the patient, two or three good blood films for staining were taken and differential counts of leucocytes as well as the proportion of gametes to leucocytes made the following morning. By this means, and by an occasional leucocyte count, it was possible to estimate from films the number of gametes ingested by the mosquito in feeding.

ESTIMATION OF GAMETES

The estimation of the number of gametes ingested by the mosquito gives one an idea as to the grades of infection to be encountered in the mosquito, and this has indirectly a practical bearing which will be discussed later.

Leucocyte counts taken during convalescence in malarial fever generally show a number not far removed from the normal. It was possible, then, to estimate the number of gametes by counting the number met with while enumerating the leucocytes during the differential count. Such a gamete count was made from the blood of every patient bitten.

Mosquitos were weighed before biting and after biting, and the amount of blood ingested estimated in this way.

Weights of Mosquitos

	Weight.
Ce. albimana bred in laboratory, twenty-four hours old, mid-gut empty	0·0008
Ce. albimana bred in laboratory, moderate blood feeding	0·0016
Ce. albimana caught in labour-cars, some blood in mid-gut, half developed ova	0·0019
Ce. albimana caught in labour-cars, much blood and early development of ova	0·0035
Ce. albimana caught at barracks, blood in mid-gut, no development of ova	0·0018
Ce. albimana caught at barracks, blood in mid-gut, no development of ova	0·0021

The average weight of mosquitos twenty-four hours old was 0·0008 gramme, while the average weight of mosquitos from the same brood that had bitten and taken a moderate amount of blood was 0·0016 gramme. The average weight of blood ingested being then 0·0008 gramme, approximately 0·001 gramme. Assuming the specific gravity of blood in malarial fever with slight anaemia to be 1·050, then 105 : 100 : : 0·0008 : 0·000761 = the volume of 0·0008 gramme of blood. Now if there were 22 gametes per 100 leucocytes, as in Experiment 38, and 6,500 leucocytes per mm.^{3} as they were, by actual count, there would be $22 \times 65 \times 0{\cdot}761 = 1088$ gametes ingested. If, under the most favourable circumstances, there are an equal number of male and female gametes, there should have been about 1,632 zygotes in this mosquito's mid-gut after three feedings,

but as a matter of fact, there were only about fifty, showing a loss of about 97 per cent. This loss may be partly explained by an observation on the fate of gametes *in vitro* and *in vivo*, when it was noticed that fully 50 per cent. of gametes were phagocyted by polymorphonuclear leucocytes. This last is truly surprising, because this type of leucocyte in the circulating blood of man rarely plays any part in malarial phagocytosis, save in pernicious infections, when it will then engulf parasites and pigment. Usually it is the large mononuclear and endothelial cells which phagocyte malarial parasites and pigment.

In Experiment 41, blood specimens from a patient taken on admission contained 92 crescents per 100 leucocytes. Fresh preparations examined fifteen to twenty minutes after being taken, contained many pigmented extra-cellular parasites, some with quiescent pigment, others with pigment dancing in a circle around a granular centre. There were free flagella in some fields—two in one field. One flagellum was seen attached to several red blood cells and was moving with extreme violence, but without becoming detached. Quite a number of the gametes became phagocyted by polymorphonuclear leucocytes, one of the latter had phagocyted two gametes. Stained specimens showed numerous gametes within the polymorphonuclear leucocytes; one free, detached flagellum was seen. Some of the gametes were globular with linear pigment and a large chromatin dot, while others contained granular pigment, surrounding a chromatin ring, staining interruptedly and having an achromatic space on its interior and exterior.

CARE OF MOSQUITOS AFTER BITING

The mosquitos may be fed nightly or every other night from patients, or, if it is desired to ascertain the rate of development of zygotes, they are fed on dates after a single biting. The mosquitos should be kept in the biting jars with fresh food and very little water, just enough to favour oviposition and to keep the air within the jar moist, but not so much that they would be drowned.

It is very necessary that the infected mosquitos be protected from ants; otherwise valuable specimens that die during the night will be removed, with nothing but wings and legs to mark the loss.

Mosquitos may be transferred one at a time from the biting jar, and from day to day killed and dissected. Chloroform and cyanide may be used for killing. Chloroform is more conveniently used, but cyanide yields better preparations when it is desired to preserve most of the mosquito for identification, for the reason that cyanide causes the mosquitos to spread their wings.

METHOD OF EXAMINING FOR SPOROZOITES AND ZYGOTES

First it should be said that successful dissections can only be obtained with killed specimens. Mosquitos that have been dead twelve or more hours, particularly if they had fallen on the water, have become so macerated that the cells of the mid-gut or salivary glands separate and float away, it being impossible to retain the organ intact. When an examination of the salivary glands is desired, the wings and legs of the mosquito are trimmed off with a small, sharp entomological knife. The distal half of the thorax, with the abdomen, is removed by a transverse, clean cut, the mosquito being laid on a piece of white cardboard. With a small pair of forceps the proboscis is grasped, and the specimen laid on a drop of saline solution on a Stender dish cover under the dissecting microscope. The chitinous covering of the thorax, just behind the nape, is carefully slit, or torn, and the muscle organ beneath loosened slightly; then by pulling out the proboscis with one needle and holding the chitinous thoracic covering with the other, the salivary glands will be drawn out. They should be cut off from the head by a small, very sharp knife, and picked up with the point of a bright needle and placed in saline solution, 10 per cent. formalin, or films made for staining.

The sporozoites will be seen either free or in epithelial cells, or in the duct of the salivary glands; appearing as thin, slightly curved, spindle-shaped bodies, placed side by side, frequently as though matted together.

METHOD OF EXAMINING FOR ZYGOTES

The identified mosquito is laid on a piece of white cardboard, the abdomen cleanly and carefully removed by a transverse cut, just behind the thorax, and placed on a glass slide, or better, the under

surface of a Stender dish cover, with a drop of saline solution. Under the dissecting microscope with the reflector properly adjusted, the hind-gut, mid-gut, Malpighian tubules and ovaries are withdrawn by pulling carefully on the last abdominal segment with one needle and holding the first abdominal segment by a corner with another needle. The mid-gut is separated from the hind-gut and Malpighian tubules as well as possible and transferred to a slide on the point of a needle, which should be sharp and well burnished, where it may be examined in saline solution, formalin, or by other methods. When the mosquito has been dead several hours, the mid-gut cannot be withdrawn intact. In this event, it is generally necessary to split the chitinous abdomen open, and search carefully for as much of the mid-gut as may have held together. Zygotes can be detected with a low-power lens, Zeiss, 16 mm. objective and 8 and 12 oculars. If an absolute identification cannot be made at the time, all parts excepting the abdomen must be preserved intact, mounted with experiment number attached for final identification.

DESCRIPTION OF THE MALARIAL PARASITE IN THE MOSQUITO

As stated above, it was found that many gametes were phagocyted in the mosquitos' stomach (50 per cent. or more) by polymorphonuclear leucocytes. This materially diminishes the number of fertilised ookinets which reach and enter the wall of the mosquito's mid-gut. Notes from the following experiment throw some light on the changes occurring in crescents *in vitro*. The phagocytosis of crescents in the mid-gut was demonstrated separately, but films do not permit as careful study as those *in vitro*.

EXPERIMENT NO. 20.—Blood contains, December 30, 67 crescents per 100 leucocytes. Temperature 97° continuously.

DIFFERENTIAL COUNT OF LEUCOCYTES

Polynuclear	68	
Large mononuclear	4	
Small mononuclear	20	67 crescents per 100 leucocytes.
Eosinophil	7	
Mast	1	

In the fresh blood film, when it is drawn, numerous crescents are seen, but within five to ten minutes half the number of crescents have become vesicular and their pigment dancing; several have thrown out flagella, which are still attached after fifteen to twenty minutes. These vesicular gametes have become phagocyted by polymorphonuclear leucocytes; some of them at this period have parted with their flagella, because several free flagella were detected in the film. Stained preparations fixed immediately upon drawing the blood show nothing but crescentic forms, but films that have been kept moist from five to ten minutes before staining show that a very marked change has taken place in the crescents, many of them becoming either globular or irregular and distorted. One film fixed after ten minutes, and stained, showed nine crescentic forms, eight globular forms, with discrete pigment and several chromatin dots, some of which are certainly microgametes, and eleven irregular forms, as though becoming globular, or as crescents losing their stiff outline, and becoming flexible.

After fertilisation the microgametocyte becomes elongated (wandering vermicule, ookinet). Illustrations of wandering vermicules (after Grassi) are very much like the eleven irregular forms seen in the blood just described. These, then, may represent fertilised gametes.

The fertilised gamete, or ookinet, if it be not phagocyted, has abundant time to wander out of the blood clot and reach the gut wall, for the blood-meal of the mosquito is usually not expelled until after twenty-four hours.

The earliest form of the malignant tertian zygote was detected in the wall of the gut after the expulsion of the blood-meal, or after two and a half days. Satisfactory dissections and examinations cannot be made until the blood-meal has been expelled; consequently, after several trials, sixty hours after a feeding was the earliest period at which a search was made for zygotes.

In Experiment No. 204, a specimen of *Ge.* (?) *tarsimaculata* was killed sixty-five hours after a single feeding from a patient whose blood contained ten crescents per 100 leucocytes. Upon examination there were about fifty zygotes in the mid-gut. They were slightly oval in outline, with closely clumped quiescent pigment, and very little cytoplasm showing beyond the pigment, the diameter being

about 5 μ. The zygotes become larger each day, though they do not always appear to grow at equal pace.

This variation in size may depend on location in the gut wall and its relation to nutrition. On the whole, however, the gradual increase in the size of the zygote is fairly uniform, as the following table shows:—

Experiment No.	Age of Zygote	Type of Parasite.	Size of Zygote in μ
16	4–4½ days	Simple tertian	12 × 16·5
16	4–4½ ,,	,, ,,	17 × 20
32	5 ,,	,, ,,	19·5
16	8½–9 ,,	,, ,,	48, 54
16	8½–9 ,,	,, ,,	48 × 54, 55·5 × 66 (Sporoblasts formed)
18	Sporozoites in salivary glands.	Smple tertian	
204	65 hours	Malignant tertian	5
9	60 ,,	,, ,,	9 × 11·25
41	2½ days	,, ,,	9 × 10·5
38	3 ,,	,, ,,	7 × 10
43	3 ,,	,, ,,	7 × 10
41	3½ ,,	,, ,,	12 × 15
38	4 ,,	,, ,,	12 × 15
33	3¾–4¾ ,,	,, ,,	12 × 13·5
42	4¾ ,,	,, ,,	21
36	5 ,,	,, ,,	21
11	6½ ,,	,, ,,	22, 28
38	7 ,,	,, ,,	45 × 57 (Contains sporoblasts)
13	7½ ,,	,, ,,	32 × 40
13	8½ ,,	,, ,,	39
36	11 ,,	,, ,,	Sporozoites in salivary glands
17	12½ ,,	,, ,,	30 (No sporozoites in salivary glands)

Measurements were made from fresh preparations in 10 per cent. formalin-saline under cover-slip with ocular micrometer (Zeiss).

The zygotes, as will be seen from the foregoing table, generally assume an ovoidal shape, one diameter being a little longer than the other. As they increase in size they become more vesicular, the periphery assumes a definite rim-like contour, and the pigment becomes more and more scattered, discrete, and ultimately, inconspicuous. During the first sixty hours the pigment is tightly clumped, but as the zygote becomes larger and vesicular, the pigment usually spreads out in lines or belts, sometimes in small clumps, or occasionally scattered and discrete. In Experiment No. 38 a specimen of *Ce. albimana* contained about fifty malignant

tertian zygotes, three and a half days old (12 × 12 μ), in its mid-gut; the pigment was in the form of linear rods, and almost every zygote had a zonal arrangement of its pigment. The zone of pigment was made up of rods, end to end, each rod separated by a small space; sometimes the zonal pigment was concentric with the periphery of the zygote, sometimes at right-angles to the plane presented to the observer, but apparently always peripheral.

In Experiment No. 33, a specimen of *Ce. albimana* contained twelve to fifteen malignant tertian zygotes, three and three-quarter to four and three-quarter days old, 12 × 13·5 μ in size, mostly oval in outline. The pigment was bronze in colour, and in clumps, never in lines or belts. In experiment No. 36 a specimen of *Ce. albimana* contained malignant tertian zygotes five days old, 21 μ in diameter, with the pigment present in clumps. On counting the number of pigment rods in a zygote, and in crescents, it is evident that conjugation of gametes does not occur, for there is the same amount of pigment in each instance. In Experiment No. 42, a specimen of *Ce. albimana* contained several malignant tertian zygotes of three ages (three bitings from the same patient). Five or six zygotes were between one and a half and two and a half days old, and their pigment, of course, tightly clumped. One zygote, 21 μ in diameter, was globular and contained three clumps of pigment, while in several others of the same age the zygotes were oviform and the rods of pigment were arranged in pairs, scattered irregularly throughout the zygote, each one containing thirteen or fourteen rods of pigment.

In most of the zygotes, excepting the very young or very old forms, the pigment was arranged in lines or belts, but not uncommonly zygotes were seen of equal age in the same specimen, some with belts of pigment, and some having it in clumps.

The larger zygotes, containing sporoblasts, showed very little pigment. This is collected in one clump, usually near the periphery. The pigment is probably not destroyed nor extruded, but is partly obscured by the greater size of the zygote.

Some of the smallest zygotes contained dancing pigment, and in the larger zygote, with zones of linear pigment, the zone could be seen to change its position very slowly, but in these instances the pigment was not dancing.

When the zygotes reach the size of 39 × 45 μ, or 45 × 57 μ (seven to eight and a half days) a fine reticulum could be seen outlining the sporoblastic chamber; while others were dotted throughout with coarse round granules, the sporoblasts.

The capsule of the zygote ruptures about the eleventh day, the sporozoites making their way to the salivary glands, and leaving the collapsed, wrinkled, disc-shaped envelopes behind in the outer layer of the mid-gut wall. The mechanism of the passage of the sporozoites into the salivary glands is not known, but these glands are more or less filled with sporozoites after the eleventh day.

The simple tertian zygote differs from the malignant tertian; first in the rapidity of its development, attaining a slightly greater size in a given time. In the younger zygotes the pigment is arranged in clumps or lines and slowly changes its position. The pigment may be clumped and dancing within vacuolated spaces of the zygote. In Experiment No. 16, tertian zygotes in *Ce. albimana* are 12 to 16·5 μ in diameter four to four and a half days after biting. The capsule of the zygote was well defined, but the zygote was apparently slightly larger than a malignant tertian zygote of the same age, and its cytoplasm was more coarsely granular than that of the malignant tertian parasite. The pigment is coarse and in clumps, not lines, and is not motile. In another specimen of *Ce. albimana*, in the same experiment, the pigment was arranged in two lines near the periphery of the zygote. Nine days after the first biting, one *Ce. albimana* with half mature ova, contained six to eight zygotes, full of faintly refractile, equally sized sporoblasts, 3 μ in diameter. These zygotes were 48 μ in diameter. Four or five zygotes were seen partly projecting from the outer wall of the gut. They were 48 by 54 μ and 58·5 by 66 μ. No pigment could be detected, but here and there faint clusters in groups, having a linear arrangement, indicating the development of sporozoites from sporoblasts. In this experiment the zygotes nearly reached maturity in nine days.

In Experiment No. 18, simple tertian sporozoites were found in only one of five acini of the salivary glands in a specimen of *Ce. albimana* eleven and a half days after the first biting. Four other acini were in plain view, but were entirely devoid of sporozoites. The sporozoites in the infected acinus were distributed in the lumen of the duct and in several of the distended vesicular epithelial cells, several

hundred being present, their long axis being generally parallel with the duct.

In Experiment No. 36, malignant tertian sporozoites were found after the eleventh day in the red-stained hyaline salivary acini on both sides of the body in large numbers. Very few in the faintly stained, larger vacuolated acini, and none in the acini containing colloid globules; but the duct of the latter contained sporozoites, which were generally matted together.

Table showing data in relation to infected and non-infected mosquitos:—

Experiment No.	No. and species of mosquito	Type of parasite	No. of gametes	Dissected: days after biting	No. of zygotes present	Controlled by *Ce. albimana*	Date of experiment	No. of patient	Ova developed
								No. 9	
2	1 M.*	?	5	3	0	No	Oct. 13	18 B.	?
3	1 A.	E.A.†	10	3½	0	Yes	,, 19	47496	No
3	1 A.	E.A.	10	3½	5	Yes	,, 19	47496	Yes
3	1 A.	E.A.	10	4½	0	Yes	,, 19	47496	No
3	1 A.	E.A.	10	4½	0	Yes	, 19	47496	No
4	1 P.	E.A.	14 + −	5	5+ −	No	,, 27	48132	No
4	1 P.	E.A.	24 + −	5	31	No	,, 27	48132	No
5	1 M.	E.A.	3·5	4½	0	No	Nov. 10	48509	No
5	1 M	E.A.	3·5	4½	0	No	,, 10	48509	No
5	1 M	E.A.	3·5	4½	0	No	,, 10	48509	No
5	1 M.	E.A.	3·5	4½	0	No	,, 10	48509	No
6	1 M.	E·A.	16	3½	0	Yes	,, 11	48987	Yes
6	1 M.	E.A.	16	3½	0	Yes	,, 11	48987	No
6	1 M.	E.A.	16	3½	0	Yes	,, 11	48987	No
9	1 P.	E.A.	27	2½	0	No	,, 24	48987	No
9	1 P.	E.A.	27	2½	6 or 7	No	,, 24	48987	No
10	1 A.	E.A.	12	4+ −	Numerous	Yes	,, 27	48987	No
11	1 A.	E.A.	8	7	0	Yes	,, 30	48987	No
11	1 A.	E.A.	8	7	Present	Yes	,, 30	48987	No
11	1 A.	E.A.	8	7	168	Yes	,, 30	48987	No
12	1 P.	E.A.	8	2½	0	Yes	,, 30	48987	No
13	1 P.	E.A.	20	3½	0	Yes	Dec. 4	48987	No
13	1 P.	E.A.	20	3½	6 or 7	Yes	,, 4	48987	No
13	1 A.	E.A.	20	7½	3	Yes	,, 4	48987	Slightly
13	1 A.	E.A.	20	8½	Numerous	Yes	,, 4	48987	No
13	1 A.	E.A.	20	8½	Numerous	Yes	,, 4	48987	No
16	1 A.	Tert.	3+	4	6 or 8	Yes	,, 21	51147	No
16	1 A.	,,	3+	4½	20+ −	Yes	,, 21	51147	No
16	1 A.	,,	3+	8½	6 or 8	Yes	,, 21	51147	Yes
16	1 A.	,,	3+	8½	4 or 5	Yes	,, 21	51147	?
18	1 A.	,,	3	11½	1	Yes	,, 27	51431	Yes
32	1 A.	,,	17 + −	4¾	2	Yes	Jan. 25	53343	Yes
32	1 A.	,,	17 + −	4¾	0	Yes	,, 25	53343	Yes
32	1 Ag.	,,	17 + −	4¾	0	Yes	,, 25	53343	Yes
32	1 P.	,,	17 + −	4¾	0	Yes	,, 25	53343	Partly
32	1 P.	,,	17 + −	4¾	0	Yes	,, 25	53343	,,

Experiment No.	No. and species of mosquito	Type of parasite	No. of gametes	Dissected: days after biting	No. of zygotes present	Controlled by *Ce. albimana*	Date of experiment	No. of patient	Ova developed
32	1 P.	Tert.	17 + −	4¾	0	Yes	Jan. 25	53343	Partly
32	1 P.	,,	17 + −	4¾	0	Yes	,, 25	53343	Yes
33	1 A.	E.A.	11	4¾	12-15	Yes	,, 26	H.G.	Yes
33	1 A.	E.A.	11	4¾	12-15	Yes	,, 26	H.G.	Yes
34	1 P.	E.A.	4	4½	0	No	,, 27	H.G.	No
35	1 Ag.	E.A.	8	2½	0	No	,, 28	53499	No
36	1 A.	E.A.	27	5	Present	Yes	,, 30	53499	Yes
36	1 P.	E.A.	27	5	0	Yes	,, 30	53499	No
36	1 P.	E.A.	27	6	0	Yes	,, 30	53499	Yes
36	1 P.	E.A.	27	6	0	Yes	,, 30	53499	Yes
36	1 A.	E.A.	27	11	Present	Yes	,, 30	53499	Yes
36	1 A.	E.A.	27	13½	,,	Yes	,, 30	53499	—
37	1 P.	E.A.	29	3	0	Yes	Feb. 1	53742	No
37	1 P.	E.A.	29	3	0	Yes	,, 1	53742	No
37	1 P.	E.A.	29	3½	0	Yes	,, 1	53742	Yes
37	1 P.	E.A.	29	3½	0	Yes	,, 1	53742	Slightly
37	1 P.	E.A.	29	3½	0	Yes	,, 1	53742	,,
38	1 A.	E.A.	29	2¾	Present	Yes	,, 1	53742	No
38	1 A.	E.A.	29	3½	Many	Yes	,, 1	53742	Slightly
38	1 A.	E.A.	29	6¾	Numerous	Yes	,, 1	53742	Yes
38	1 P.	E.A.	29	11½	0	Yes	,, 1	53742	No
38	1 A.	E.A.	29	11½	Many	Yes	,, 1	53742	Slightly
41	1 A.	E.A.	92	3½	Numerous	Yes	,, 5	53937	Yes
41	1 A.	E.A.	92	8½	?	Yes	,, 5	53937	?
41	1 A.	E.A.	92	10½	—	Yes	,, 5	53837	—
44	1 P.	E.A.	3·5	1½	0	No	,, 14	53937	No
44	1 M.	E.A.	3·5	2¾	0	No	,, 14	53937	No
44	1 M.	E.A.	3·5	4½	0	No	,, 14	53937	Yes
44	1 M.	E.A.	3·5	4½	0	No	,, 14	53937	No
44	1 P.	E.A.	3·5	4½	0	No	,, 14	53937	Slightly
46	1 A.	E.A.	2	1½	0	Yes	,, 17	53937	No
47	1 P.	E.A.	½	3½	0	Yes	,, 19	53937	No
47	1 P.	E.A.	½	3½	0	Yes	,, 19	53937	No
47	1 A.	E.A.	½	3½	0	Yes	,, 19	53937	Slightly
47	1 A.	E.A.	½	3½	?	Yes	,, 19	53937	Yes
47	1 A.	E.A.	½	3½	No	Yes	,, 19	53937	Yes
47	1 A.	E.A.	½	3¾	3	Yes	,, 19	53937	No
47	1 A.	E.A.	½	3¾	4	Yes	,, 19	53937	No
47	1 P.	E.A.	½	3¾	0	Yes	,, 19	53937	No
47	1 M.	E.A.	½	3¾	0	Yes	,, 19	53937	No
47	1 A.	E.A.	½	3¾	0	Yes	,, 19	53937	Slightly
47	1 M.	E.A.	½	3¾	0	Yes	,, 19	53937	No
47	1 P.	E.A.	½	3¾	0	Yes	,, 19	53937	No
47	1 P.	E.A.	½	3¾	0	Yes	,, 19	53937	No
47	1 P.	E.A.	½	3¾	0	Yes	,, 19	53937	No
42	1 A.	E.A.	10	1½	0	Yes	,, 6	53742	No
42	1 A.	E.A.	10	1½	0	Yes	,, 6	53742	No
42	1 P.	E.A.	10	1½	0	Yes	,, 6	53742	No
42	1 A.	E.A.	10	3¾	Several	Yes	,, 6	53742	Yes
42	1 M.	E.A.	10	3¾	0	Yes	,, 6	53742	Yes
42	1 A.	E.A.	10	4½	Present	Yes	,, 6	53742	Yes
42	1 A.	E.A.	10	4½	,,	Yes	,, 6	53742	Yes
42	1 A.	E.A.	10	5¾	Many	Yes	,, 6	53742	Yes
42	1 A.	E.A.	10	5¾	,,	Yes	,, 6	53742	Yes
42	1 A.	E.A.	10	5¾	3 or 4	Yes	,, 6	53742	Yes

Experiment No.	No. and species of mosquito	Type of parasite	No. of gametes	Dissected: days after biting	No. of zygotes present	Controlled by *Ce. albimana*	Date of experiment	No. of patient	Ova developed
42	1 A.	E.A.	10	$5\frac{3}{4}$	3 or 4	Yes	Feb. 6	53742	No
43	1 A.	E.A.	5	$2\frac{3}{4}$	5	Yes	,, 8	53742	No
43	1 M.	E.A.	5	$2\frac{3}{4}$	0	Yes	,, 8	53742	No
47	1 P.	E.A.	$\frac{1}{2}$	$5\frac{1}{2}$	0	Yes	,, 19	53937	No
47	1 M.	E.A.	$\frac{1}{2}$	$5\frac{1}{2}$	0	Yes	,, 19	53937	No
47	1 Ag.	E.A.	$\frac{1}{2}$	$5\frac{1}{2}$	0	Yes	,, 19	53937	No
47	1 Ag.	E.A.	$\frac{1}{2}$	$5\frac{1}{2}$	0	Yes	,, 19	53937	No
47	1 M.	E.A.	$\frac{1}{2}$	$5\frac{1}{2}$	0	Yes	,, 19	53937	No
202	1 P.	?	0 ?	4	0	No	Aug. 24	Bed 90	Yes
202	1 T.	?	0 ?	4	0	No	,, 24	Bed 90	Yes
204	1 A.	E.A.	5 + −	3	40	Yes	,, 28	63472	Yes
204	1 T.	E.A.	5 + −	4	Many	Yes	,, 28	63472	No
204	1 A.	E.A.	5 + −	9	Many	Yes	,, 28	63472	Yes
209	1 T.	E.A.	5 + −	2	20	Yes	Oct. 5	65343	Yes
209	1 T.	E.A.	5 + −	2	22	Yes	,, 5	65343	Yes

*A. = *albimana*
M. = *malefactor*
T. = *tarsimaculata*
P. = *pseudopunctipennis*
Ag. = *argyrotarsis*

†E.A. = malignant tertian
Tert. = simple tertian

Notes and Conclusions from the foregoing Table

Species	Number	Infected	Percentage infected
Arr. (?) malefactor	17	0	0·0
A. pseudopunctipennis	31	4	12·9
Ce. albimana	48+2	34+2	70·2
**Ce. argyrotarsis*	4	0	0·0
Ce. tarsimaculata...	5	3	60·0

* A naturally-infected specimen of this species has been found in barracks.

Out of several hundred mosquitos used in the biting experiments 107 gave satisfactory dissections, or paraffin sections, and it was determined that:—

70·2 per cent. of *Ce. albimana* became infected;
60 per cent. of *Ce. (?) tarsimaculata* became infected; and
12·9 per cent. of *A. pseudopunctipennis* became infected;

while none of *Arr. (?) malefactor* became infected, although several were placed in jars with *Ce. albimana* and bit at the same time persons from whom the specimens of *Ce. albimana* became infected.

It is concluded from this series of experiments that *Ce. albimana*, the common white hind-footed mosquito—an extremely hardy, rapidly developing, adaptable mosquito—is the transmitter of malignant tertian and of simple tertian malarial fever in the Canal Zone at this time. Specimens of this species infected with simple tertian parasites became infective between nine and eleven and a half days after the first feeding. When infected by malignant tertian parasites, sporozoites appeared in the salivary glands as early as the eleventh day in some mosquitos, and later than twelve and a half days in others.

Ce. (?) tarsimaculata appears to be as susceptible to the malignant tertian parasite as *Ce. albimana*, and no doubt if a favourable opportunity had presented it would have been found that *Ce. (?) tarsimaculata* would have been equally susceptible to tertian malaria.

A. pseudopunctipennis is only slightly concerned in the transmission of malarial fever, if at all, not only from the fact that only four out of thirty-one mosquitos under the most favourable conditions became infected, but from the additional fact that relatively few specimens are taken in quarters at this time.

Arr. (?) malefactor is not concerned in the transmission of malarial fever in the Canal Zone at this time.

Out of forty-one mosquitos containing malarial zygotes, seventeen showed development of ovaries or ova. A few of these, however, might have oviposited before examination.

Out of sixty negative bitings, i.e. where mosquitos of any of the species bit and failed to become infected, twenty showed development of ova; and to take the known susceptible species, *Ce. albimana* and *Ce. (?) tarsimaculata*, out of thirteen *Ce. albimana* and *Ce. (?) tarsimaculata* which failed to become infected six showed development of ova.

It would, therefore, appear that fecundation and development of ova are not necessary for the development of zygotes.

Under the most apparently favourable circumstances one or two out of the lot of susceptible Anophelines will fail to become infected, and these, no doubt, possess an active immunity toward the malarial parasite.

LIMITS OF INFECTIOUSNESS OF MAN

During the progress of the experiments it was noticed that patients were discharged after their temperature had become normal and when their peripheral blood occasionally contained more than a sufficient number of gametes to infect susceptible mosquitos. In order that a recommendation might be made for the continued treatment of these persons, it was necessary to determine, if possible, the limits of infectiousness of such individuals.

Several experiments were carried out, in which *Ce. albimana* bit patients whose gametes per leucocytes had been determined. These mosquitos were given but one blood feeding and fed subsequently on dates and raisins and then dissected. The limits were determined as being near one gamete per 500 leucocytes, or twelve gametes per $mm.^3$; but it must be understood that several factors are concerned in infections, such as number and phagocytic power of leucocytes; immunity of mosquitos, racial and individual; probable reaction of gut contents, as acid bacterial products or those from yeasts, may be inimical to the gametes; and again proportion of ♀ to ♂ gametes plays some part, besides the number of gametes ingested. In Experiments 20 and 28 patients' blood was rich in crescents which flagellated *in vitro*, and in the mosquito's stomach, yet mosquitos never could be infected from the patients.

Persons with more than twelve gametes per $mm.^3$ must be regarded as gamete carriers, and, of course, should not be discharged from hospital nor should treatment be discontinued until gametes have been reduced well below the limits of infectiousness. This destruction and prevention of the development of the sexual forms of the parasite in man is a matter generally overlooked, but is of the greatest importance in delimiting malaria, and it may be accomplished by appropriate quinine treatment of all gamete carriers; by quinine treatment to destroy latent malaria, and by periodical blood examination of labourers and others in quarters where there is a high malarial rate. For the detection of gamete carriers and latent malaria, in order to carry out appropriate treatment, 30 grains of quinine sulphate in solution daily is an efficient dosage for the purpose required.

EXPERIMENT No. 47.—February 19, 1909, 8 p.m., jar containing *Ce. albimana, A. pseudopunctipennis* and *Arr. (?) malefactor* was applied to No. 53,937, who was receiving 30 grains of quinine daily when his blood contained one crescent to 200 leucocytes = approximately + 30 gametes per mm.³. The mosquitos were given but one blood feeding and subsequently fed on raisins and dates.

Upon dissection on February 23-5 :—

9 *A. pseudopunctipennis* were not infected
4 *Arr. (?) malefactor* ,, ,,
2 *Ce. argyrotarsis* ,, ,,
3 *Ce. albimana* ,, ,, while
1 *Ce. albimana* contained three zygotes, and
1 *Ce. albimana* contained four zygotes.

EXPERIMENT No. 31.—January 25, 1909, a jar containing *A. pseudopunctipennis, Ce. argyrotarsis* and *Ce. albimana* were fed from 53343, tertian malaria fever, whose blood contained four gametes per 100 leucocytes.

Upon dissection, January 30 :—

3. *A. pseudopunctipennis* were not infected;
1 *Ce. argyrotarsis* was not infected;
1 *Ce. albimana* was not infected; while
1 *Ce. albimana* contained two zygotes.

NOTES ON THE BIONOMICS OF SOME OF THE ANOPHELINES STUDIED

The period of incubation of the ova of *Ce. albimana, A. pseudopunctipennis* and *Arr. (?) malefactor* was estimated as about thirty-six hours under the laboratory conditions, the temperature of the air and water ranging between 78° and 82° daily, an eastern window exposure with direct sunlight for three or four hours in the morning, and diffused sunlight the remainder of the day. The eggs are laid in the geometrical patterns usually seen, and at first are creamy-white colour, becoming in a few hours quite black, with white lateral air chambers.

The larval period varies with the species, food, efficient temperature, sunlight and environment; for example, ova of *Arr. (?) malefactor* and *Ce. albimana* of about the same age were exposed in the same breeding tank to an identical environment, food, water, air, and sunlight; but when *Ce. albimana* had pupated the larvae of *Arr. (?) malefactor* were only half grown. In one experiment *Ce. albimana* larvae pupated within twelve days, while *Arr. (?) malefactor* required sixteen to twenty days. Subsequent examinations of water from *Arr. (?) malefactor* pools and from the intestinal tracts of *malefactor* larvae indicate that the latter

prefer shady pools in which chlorophyll-bearing algae, the chief food of *Ce. albimana* larvae, are relatively absent.

Ova of *Ce. albimana*, still creamy-white in colour, were placed in a breeding tank exposed to the morning sun on December 3; temperature of the water, 28·5 to 30° C. (78° to 82° F.). Of these ova five became larvae and pupated December 14-15. These pupae became imagines during the night of 16-17, making the period from ovum to imago about thirteen and a half days. Under these conditions they did not get as much sunlight as they would have received outside. Sunlight and the abundance of algae undoubtedly play a great part in the duration of the period of incubation. It should be added that these five mosquitos, two males and three females, were placed in a biting jar the morning they emerged—December 17, and that same evening each one of the three females bit and drew blood at once when applied to the arm of a patient. In this instance mosquitos bit when not more than twenty-hour hours old.

Into the same tank were placed sixty-eight larvae of *Arr. (?) malefactor* from ova laid December 3; one-third of the ova hatched the morning of December 5, the approximate age of the ova, or the period of incubation, was thirty-six hours. The tank was supplied with algae, spirogyra, and the water aerated. It was noticed very soon that the *malefactor* larvae did not mature as rapidly as the *albimana* larvae did; when specimens of the latter were full grown the *malefactor* were only one-third or half grown, and the *malefactor* larvae did not pupate until sixteen to twenty days after hatching.

HARDINESS OF *CE. ALBIMANA*

This mosquito is well fitted for the purpose of transmitting malarial fever. It is the commonest species here at the present time, outnumbering all others, excepting, possibly, *A. pseudopunctipennis*, which latter species is not very hospitable to the malarial parasite.

It breeds in a great variety of locations; besides the customary pools and margins of streams, collections of rainwater, during the dry season it may be found in the stinking water of sewage streams, brackish marshes, running streams, meadows, muddy pools, old crab holes, and in shady *malefactor* pools and river margins.

It matures more rapidly than either *A. pseudopunctipennis* or *Arr. (?) malefactor.*

It outlives *pseudopunctipennis* and *malefactor,* in confinement at any rate, which is a proof of its ability to persist, for when these species are placed in one breeding jar *malefactor* dies quickly, next follow *pseudopunctipennis,* while specimens of *albimana* survive for days longer.

DURATION OF LIFE OF MALES AND FEMALES IN CAPTIVITY

Male specimens of *A. pseudopunctipennis* which have been kept in breeding jars with females, and supplied with raisins, dates and water, have lived for eighteen days. Male specimens of *Ce. albimana* have lived twelve and a half days. On the other hand, a virgin specimen of *Stegomyia calopus* has lived for one hundred and ten days.

When virgin Anophelines have been given one or two blood meals two or three days after emerging they have lived as long as sixteen days. This is in rather striking contrast with *Stegomyia calopus,* which under similar conditions lives months to the Anophelines weeks. Several specimens of *S. calopus* virgins still under observation have lived for several weeks, and have oviposited as late as sixty days after emerging, though never in contact with males.

MUSICAL NOTE OF MOSQUITOS

The characteristic musical note of Anophelines is caused by the vibration of the proboscis, as the following observation indicates:—

A specimen of *Arr. (?) malefactor* was badly wet and sprawled; upon placing her upon a piece of filter paper and touching or approaching her proboscis the latter vibrated visibly, and emitted the characteristic high-pitched note; the wings were at rest, being stuck to the paper. This was verified again and again. Later I picked up a slightly water-sprawled, infected mosquito for dissection and held it over a few drops of chloroform; both wings were seen to vibrate rapidly as in flight, but noiselessly, while holding the mosquito by the last abdominal segment and touching one wing at its tip the opposite wing would immediately stop

vibrating. Upon releasing the wing, both would vibrate noiselessly as before. The noise of the mosquito is due, then, to the vibration of its proboscis, and the wing vibration is dependently and automatically co-ordinated.

RELATIVE VALUE OF DIFFERENT FOODS FOR ANOPHELINES

After trial with bananas, I found that raisins and dates with water furnished the best food for Anophelines in confinement. A greater number of males and females may be kept alive during the few days after emergence if fed with fresh bananas, raisins or dates; but females fed on this diet daily would not bite with alacrity. Mosquitos fed daily or on alternate days on human blood made better dissections after the digestion and evacuation of their meal than those fed on bananas. The former would be fairly free from yeast and bacteria, and the mid-gut and appendages would not disintegrate so rapidly after death. If the mosquitos were fed alternately on bananas and blood they would frequently die with an undigested hard mass of blood in the mid-gut, which must have been either impossible to digest or evacuate. The best method of feeding infected mosquitos would seem to be to feed two or three times on a patient favourable for infection, and subsequently with raisins, dates and water. Then, too, the acid contents of the mid-gut after banana feeding, with its fermentation, may interfere with the infection of mosquitos by malaria. In Experiments Nos. 20 to 28 a patient having at times sixty-seven crescents per one hundred leucocytes in his peripheral blood was bitten by four varieties of mosquitos, *Ce. albimana*, *Ce. argyrotarsis*, *A. pseudopunctipennis* and *Arr. (?) malefactor*, over a period of thirty-five days. During the intervals between biting, however, the mosquitos were fed on bananas, and none became infected.

IDENTIFICATION OF LARVAE

It must be evident that identification of Anopheline larvae in the field is of considerable importance, and in this region the malaria-transmitting Anophelines can be readily identified by certain anatomical characters. I have made no attempt to determine in

detail all the anatomical characteristics of Anopheline larvae of this region; that has been done for some species by Knab. The chief anatomical differentiating larval characters of the common Anophelines of this region are these:—

Ce. albimana or white-footed group	Palmate hairs on all abdominal segments and sometimes on postero-external angle of thorax	Antennae without a tuft of hairs
A. psendopunctipennis group	Palmate hairs on third, fourth, fifth, sixth, seventh abdominal segments, but none on the first and second. On the latter two, however, there is a rudimentary stalked tuft.	Antennae without a tuft of hairs.
Arr.(?) malefactor, or spotted-legged group	No palmate hairs on first and second abdominal segments, but palmate hairs on all remaining segments.	Antennae with a tuft of hairs.

These characters are very striking and sharply separate the groups, thus separating the malaria-transmitting *Ce. albimana* group from other varieties. With care it is frequently possible, even in muddy water, from an examination of the indentations of the surface film caused by the palmate hairs, to at once determine the presence or absence of members of the *albimana* group. In the latter group there is no break in the indented film, but in the two former groups there is a well defined non-indented break in the film, due to the lack of palmate hairs on the first and second abdominal segments.

The size of the palmate hairs on the postero-lateral angle of the thorax and the presence of these hairs on the thorax, first and second abdominal segments, is subject to some variation. It would seem that the white hind-footed group are undergoing some variation with regard to the size and location of these hairs and apparently they are becoming rudimentary or vestigial on the thorax and first abdominal segment.

FOOD OF ANOPHELINE LARVAE

The generally separate and distinct breeding places of *Arr. (?) malefactor* and *Ce. albimana*, for instance, naturally suggest that their food might also be different. Dissection of specimens of *A. pseudopunctipennis* and *Ce. albimana* in all instances discloses much green algae in the intestinal tract, while

specimens of *malefactor* usually contains much dark brown, unidentified vegetable fibres, brown organic débris, and conidia resembling those of *Pestalozia trunculata*, *Leptosporum bifurcatum*, *Paramoecia*, etc., *Rotifer vulgaris*. This indicates that *Ce. albimana* and *A. pseudopunctipennis* prefer sunny pools, while *Arr. (?) malefactor* prefers shady ones, where there is a relative absence of chorophyll-bearing forms. This is not intended as an absolute statement, because during the dry season, and in certain situations, *malefactor* and *albimana* will be found together in the same streams or pools, but it indicates certain different tendencies in the respective species.

BLOOD FEEDING NECESSARY FOR ANOPHELINES

A blood meal seems to be necessary for the development of the ova of Anophelines. In Experiment No. 40, to determine this point, male and females, *Ce. albimana* and *A. pseudopunctipennis* were placed in a breeding jar and fed on vegetable food and water daily, but they received no blood meals. Upon dissection of females as they died, none showed any development of the ovaries.

PARTHENOGENESIS

If, however, there be given one blood meal the ova may develop even in virgins kept out of contact with males. In the latter instance (with *Stegomyia calopus*) the ova have never developed into larvae.

EXPERIMENT.—Virgin Anophelines bred out from single isolated pupae were transferred to one jar entirely out of contact with males. There were three *Ce. albimana* and two *A. pseudopunctipennis*. When applied to the arm all the *Ce. albimana* drew blood; neither of the *A. pseudopunctipennis* would bite. The following day one *A. pseudopunctipennis* died, but the remaining one, with two *Ce. albimana* bit again upon application to the arm. These were added to another jar of virgin females, *Ce. albimana* and *A. pseudopunctipennis*, most of which drew blood readily. Upon dissection none of the *A. pseudopunctipennis* showed any development of the ovaries. One *Ce. albimana*, about fourteen days old, contained ova 0·48 mm. long. The single spermathecae of the mosquitos were examined, and in no instance contained spermatozoa. The ova would not develop into larvae in water, and upon microscopic examination were found to contain finely granular food material, but no partly developed larvae, such as would be seen in fertile ova of this size.

A further experiment with *Stegomyia calopus* indicates that whereas ova may develop in size in the ovaries of unfecundated virgins, they are always sterile and never contain partly developed larvae, but granular, undifferentiated protoplasm or food material.

A. November 11 a jar containing five female *Stegomyia calopus* which had been separated individually as pupae, and always out of contact with males emerged November 9. They were applied to the arm and three bit and drew blood; they were not as voracious as Anophelines, but behaved with the caution and timidity characteristic of *Stegomyia*. After fourteen days these mosquitos had not oviposited. Two were dissected and the spermathecae found to be free from spermatozoa, but their ova were well developed, 0·560 mm. in length, 0·184 mm. in width.

B. Bred out nineteen virgins, *Stegomyia calopus,* in the same manner, from isolated pupae. Upon applying to arm, sixteen out of nineteen bit and apparently drew blood, but there was no sensation of stinging. The next day when applied, only three mosquitos bit. These may have been the ones that did not bite the day before. Thirty-seven days after the first biting, eight of these virgins were living, the dead ones having become water-sprawled. Forty black ova were found in the water dish this morning.

41 days after the first biting six ova were found in the dish;

54 days after the first feeding one dead female was found, and upon dissection contained no ova;

69 days after the first feeding a dead female was found to contain twenty well-developed ova, 0·560 mm. in length;

61 days after first feeding forty-three black ova were found in the dish, completely developed, 0·720 mm. in length, 0·240 mm. in width;

but none of these ova developed later into larvae. One hundred and four days after the first feeding the remaining female is still living.

EFFECT OF SALT AND SEA WATER ON ANOPHELINE LARVAE

In general, the effect of an irritating, toxic, or otherwise unusual fluid on mosquito larvae is to hasten pupation. A number of experiments were tried with sea water, salt water and solutions of the heavy metals, and in most instances in the more concentrated solutions, when the larvae were not killed within twenty-four hours, they pupated, and occasionally the period of pupation was shortened; so that if, for instance, in a district sea water were used as a larvacide the first effect would be to hasten pupation and thus increase the number of Anophelines in the district, and if later the sea water became diluted by rain, several species of malaria-transmitting Anophelines might breed in it without difficulty, notably *Ce. albimana* and *Ce. tarsimaculata*. On this account sea water could not be used with any degree of success as a larvacide for Anophelines, except in large quantities and in certain locations.

CHLORINE CONTENTS OF NATURAL WATERS IN WHICH MOSQUITO LARVAE HAVE BEEN TAKEN, AND IN SOME INSTANCES BRED OUT

	Per cent. of Sodium Chloride
A. pseudopunctipennis	0·00165
Ce. albimana	1·93
Anopheles (Sp.)	0·65
Ce. albimana	1·165
Ce. albimana	0·255
Arr. (?) *malefactor*	0·16
Ce. albimana	0·16
Arr. (?) *malefactor*	0·00002
Ce. albimana	0·00125
Ce. tarsimaculata	0·16
—	0·21
—	0·63
—	1·02
Ce. albimana	0·02
A. pseudopunctipennis	0·02
Stegomyia calopus	0·26
Culex (Sp.)	0·057
Aedes taeniorbynchus	2·20

Sea water taken from Panama Bay contained 3 per cent. of sodium chloride, a sample from Simon Bay (Atlantic) contained 3·17 per cent., so that it will be seen from the foregoing table that some Anophelines, under stress of circumstances, may breed in very brackish water.

EXPERIMENTS WITH LARVACIDES

A number of experiments were carried out for the purpose of obtaining a cheap and efficient preparation for destroying mosquito larvae. Crude petroleum oil was frequently too viscid to have a spreading power of the highest efficiency. When mixed with crude carbolic acid, however, its spreading powers were increased.

Much of the crude carbolic acid supplied had been found upon analysis to consist chiefly of inert neutral oils with a small proportion, 5 per cent. to 10 per cent., of tar acids, and as this crude acid was used extensively as a disinfectant, experiments were conducted for the purpose of utilising if possible this crude carbolic acid as a disinfectant and larvacide. It was found that crude carbolic acid, having a specific gravity not greater than 0·96 or 0·97 and containing about 20 per cent. of phenols or tar acids, when made into a soap with common resin and an alkali yielded a product which was an ideal larvacide, having

excellent diffusing and toxic powers, and at the same time it was a very efficient germicide. It diffused perfectly with water, forming a milky emulsion very destructive to mosquito larvae, and having a germicidal value of, or greater than, that of pure carbolic acid, or a Rideal-Walker co-efficient of one to two. In this way a very valuable larvacide and disinfectant, miscible with water, was produced from a very inferior disinfectant.

The larvacidal powers when tried with Culicine and Anopheline larvae varied slightly with the quality of the crude carbolic acid, but an average result is as follows:—

Dilution 1 to 1000—Culicine larvae, dead in 5 minutes.
Anopheline larvae, half grown, dead in 5 minutes.
Anopheline larvae, full grown, dead in 10 minutes.

Dilution 1 to 5000—Anopheline larvae, half and full grown, dead in 5 minutes.
Culicine larvae, half grown, dead in 3 minutes.

Dilution 1 to 10000—Culicine larvae, half grown, dead in 64 minutes.
Anopheline larvae, young, dead in 52 minutes.
Anopheline larvae, full grown, dead in 135 minutes.

Dilution 1 to 15000—Small Culicine larvae, dead in 32 minutes.
Anopheline larvae, full grown, dead in 123 minutes.

Anopheline larvae seem to be slightly more resistant than Culicine larvae, and all pupae are more resistant to the effects of the larvacide than larvae are.

EXPERIMENTS WITH AGENTS DESTRUCTIVE TO VEGETATION, GRASS AND ALGAE

A series of experiments was carried out with the larvacide, caustic soda, arsenic and copper sulphate as to the amounts necessary in pools and lagoons to prevent the growth of vegetation and to determine the value of the resulting solutions as larvacides. Bermuda grass in sod was made into artificial ponds in large glass moist-jars and flooded with 0·5 per cent. solution of caustic soda, copper sulphate and sulphuric acid. The sod was well soaked with the chemical solution, but the grass remained vigorous in each instance. The jars were undisturbed for a period of eighteen days, when a number of Culicine larvae were introduced into the solution of the artificial pools. The larvae were killed within twenty-four hours in the pools containing copper sulphate and sulphuric acid; but those in the pool

containing caustic soda remained alive several days. It was concluded from this that none of the above chemicals could be used to advantage in killing gross vegetable matter such as grasses, and none were of special value as larvacides.

An artificial pool as above was flooded with a 0·125 per cent. solution of sodium arsenite. All but three or four of the stalks of grass were killed and overgrown with mould, the wilting effect becoming apparent in forty-eight hours. After nine days, when the grass was quite dead, several Culicine larvae were introduced into the pool and were killed after one hour's exposure. The pool was twice flushed out to rid it of arsenic salt, but the grass showed no further signs of life at the end of thirty-five days. It was concluded from this that a 0·125 per cent. solution is a valuable agent in destroying gross vegetable forms such as grass, and the resulting water in the pool remained effective as a larvacide.

The common, green, filamentous algae, *Spirogyra* and Culicine larvae were introduced into small glass jars, containing various high dilutions of copper sulphate and sodium arsenite. The results of two series of experiments showed that copper sulphate in dilutions up to 1 part in 500,000 is inimical to the growth of this alga. They become greyish-green in colour, shrunken and lose their fresh and crisp appearance. As a larvacide, however, copper sulphate is not destructive in dilutions higher than 1 in 50,000 parts. Sodium arsenite, on the contrary, seems to stimulate the growth of these algae in all dilutions between 1 in 2,500 and 1 in 25,000,000, the algae remaining green and vigorous. As a larvacide, Culicine larvae were destroyed in sodium arsenite dilutions up to 1 in 100,000. The larvacidal powers of sodium arsenite solutions in contact with green algae seem to vary within wide limits, depending probably upon the power of the algae to take the arsenic salt out of solution into its own protoplasm, thus rendering the surrounding solution less larvacidal. It is concluded from this that copper sulphate is more efficient than sodium arsenite as an algacide in high dilutions, but the arsenic salt is a better larvacidal agent. These results are in keeping with our pharmacological knowledge of the effect of copper and arsenic salts in high dilutions on animal and vegetable protoplasm. It would seem, then, that when grass and algae in pools, without outlets, are to be destroyed, sodium arsenite

would be of considerable value for this purpose, and would continue to be efficient until washed or drained out and that copper sulphate is a valuable algacidal agent for the destruction of filamentous algae, as *Spirogyra*.

In experiments with the coal tar larvacide in laboratory tanks and under actual conditions the coal tar larvacide was found destructive to grass in dilutions of 10 per cent., the grass turning brown in two or three days and drying in five to six days. When *Spirogyra* was treated with the larvacide, dilutions of 1 to 2,500 were sufficient to kill, while dilutions of 1 to 5,000 and 1 to 10,000 greatly reduced its vigour.

COMPOSITION AND SIZE OF MESH OF WIRE SCREENING

Two extremely important factors in the use of wire screening for protection against mosquitos, are, first, the size of the mesh, and secondly, the chemical composition of the wire used. In regions where it is only necessary or desirable to protect against Anophelines, a No. 16 mesh screening (sixteen holes to the inch) would answer the purpose, and where, as in this region, it is necessary to protect against some of the smaller species, such as *Stegomyia calopus*, a No. 16 mesh would be practically safe, but not absolutely so. The following experiments were conducted to determine the varieties of mosquitos which would, under stress of circumstances, pass through a No. 16 mesh wire screening. Out of several hundred mosquitos eight common species were able to make their escape through a No. 16 mesh wire.

	Sex	No. of specimens escaped
Stegomyia calopus	Males	10
" "	Females	6
Culex cubensis	Male	1
" *rejector*	Male	1
" *extricator*	Female	1
Aedes angustidittatus	Female	1
Uranotaenia lowii	Female	1

No specimens of *Ce. albimana* or *A. pseudopunctipennis* escaped through No. 16 wire mesh screen, although several hundred were tried. The methods adopted were as follows:—

A. A square wooden box, well ventilated, with fine crinoline gauze screening on two sides and glass on the other two sides, with a central replaceable partition, covered with No. 16 mesh wire screening, was constructed. Several dozen mosquitos at a time, of the above species were liberated on one side of the partition without food or water, and on the opposite side, close to the screen partition, were placed water, banana, candy, sugar and raisins as bait. Only three mosquitos out of several hundreds of several varieties passed through the No. 16 mesh partition under the conditions of the experiment. As the space including the mosquitos was about one-half of a cubic foot in volume, and as there were a few recesses in which the mosquitos could hide, an electric light bulb was hung in such a position at night that the mosquitos would be attracted by it, but this did not favour the passage of mosquitos through the screen. Tobacco fumes were passed into the mosquito compartment with a rubber bulb apparatus, and while this excited the mosquitos, did not cause any of them to escape through the screen. When a person's arm was introduced into the compartment close to the No. 16 mesh wire partition it did not induce the mosquitos to escape through the screening.

B. Next a lantern-chimney, covered on one side with fine mesh crinoline gauze and on the other side with a metal collar holding in place a piece of the No. 16 mesh wire screening, was partly filled with various mosquitos and placed near the same bait as before under a large glass bell jar. Eighteen mosquitos escaped from the chimney through the No. 16 mesh screening into the surrounding jar. The closer quarters and the absence of resting places in the chimney evidently favoured the escape of mosquitos through the wire screening. On one occasion, by passing a gust of air through the lantern chimney jar, a male *Culex* was helped through and escaped.

The conditions in the experiments were all rigid and more extreme than those under actual conditions where mosquitos are trying to enter a screened house from the open.

The chemical composition of various screening material used was investigated by Dr. R. W. Nauss, formerly of this Laboratory. Screening of excellent quality was compared with that which had deteriorated more or less rapidly, and analyses of screens and their incrustations made to determine the factors concerned in its corrosion.

In the investigation considerable attention was paid to the analyses of the efflorescence or incrustation formed on the screening for the determination of the constituents involved in the corrosion. The specimens presenting the highest degrees of deterioration furnished the largest amounts of incrustation. The deterioration of the screening is largely due to the presence of iron in the brass alloy, plus the influence of a hot, moist atmosphere.

Observations continued over a period of four years on screening made of copper and zinc with a composition nearly :—

Copper ...	84·92	89·94	84·83	88·59	95·85
Zinc	—	—	14·90	—	4·15
Iron	—	—	0·06	0·04	0·0

showed that these resist the corroding actions of a hot, moist climate much better than screening made of brass with an average composition of :—

Copper	65
Zinc	34
Iron	1 + —

and it is concluded that screening intended for use in the tropics, exposed to heat and moisture, should have a high copper content, higher than brass, and be as free as possible from the presence of iron.

VALUE OF DAILY COLLECTION AND DESTRUCTION OF LIVE MOSQUITOS CAUGHT IN BARRACKS AND QUARTERS

Abundant material was received for observations on the value of this practice. The daily catch of mosquitos from barracks of various districts would be sent alive to the laboratory. The mosquitos were transferred to breeding jars and fed on dates or raisins until their intestinal tracts were free from blood. They were then killed and examined for zygotes or sporozoites. The species examined were: *Ce. albimana*, *Ce. tarsimaculata*, *Ce. argyrotarsis*, *Arr. (?) malefactor*, and *A. pseudopunctipennis*. A number of specimens of Culicines were also examined at this time. It is noteworthy, and speaks well for the practice of these daily killings, that but one naturally infected Anopheline was found, and that one a specimen of *Ce. argyrotarsis*.

After the first forty-three negative dissections no record was kept of the total number examined, but it was in the neighbourhood of five hundred.

EFFECT OF QUININE ON THE MALARIAL PARASITE IN (*A*) THE MOSQUITO AND (*B*) MAN

A. Nearly all the infecting experiments were conducted on patients who were receiving the routine ward treatment of quinine, grains 10, *ter. die*, in solution, so that apparently quinine in these quantities has no destructive or inhibitive effect on the parasites in the mosquito because the zygotes go on to maturity and sporozoites appear in the salivary glands in from nine to eleven days.

One experiment should be mentioned, however, because the patient received no quinine for several days before the mosquitos became infected and none during the experiment, so that the mosquitos never received any quinine. One *Ce. albimana* contained the rather large number of one hundred and sixty-eight zygotes upon dissection. It should be mentioned as well that from this patient two *A. pseudopunctipennis* became infected. It may be that quinine has a slight inhibitory effect on the parasite in the mosquito's mid-gut.

B. The following tables show the gradual but steady decrease in the number of gametes in the peripheral blood during the administration of quinine, grains 10, *ter. die*, in solution, and one table shows the effect of withholding quinine.

The differential leucocyte counts are tabulated as well, and in these the relative increase in mononuclear elements—lymphocytes, intermediate, and large mononuclear cell—the latter being the chief circulating phagocytic cell in malaria.

As indicative of blood regeneration and the secondary effect on the homopoietic organs, it is interesting to note the increase in the eosinophiles.

CHART 51499

	Dec. 30	Dec. 31	Jan. 2	Jan. 5	Jan. 6	Jan. 8	Jan. 13	Jan. 15	Jan. 23	Feb. 1
Polymorphonuclear	68	65	75	81	65	61·5	65	51	53	66
Large mononuclear	4	16	6	5	6	13·5	13	11	9·5	2·5
Lymphocyte	20	13	13	11	20	16·5	11	14	18	11·5
Large lymphocyte—										
Intermediate	—	—	—	—	—	—	—	8	4	7·5
Eosinophile	7	5	5	2	8	7	11	14	14·5	12·5
Mast	1	1	1	1	1	1·5	0	2	1	0
Crescents	67	42	76	46	40	15·5	9	5	0·5	0

The table given above shows the rate of diminution in the number of gametes (crescents) by the administration of quinine, grains 10, *ter. die*, in a Spaniard, 60 years of age, on Isthmus twenty months, whose blood contained numerous crescents but no young forms, and whose temperature was 97° F. continuously. It also shows the degree of change in the proportion of eosinophiles, of increase in mast cells, very slight lymphocytosis and polymorphonuclear decrease.

Compare this with the following:—Case 48,987 of malignant tertian malarial fever, from whom quinine was withheld for twenty-four days. Spaniard, on the Isthmus three months, temperature normal on admission.

1908.	Nov. 11	Nov. 24	Nov. 27	Nov. 30	Dec. 4
Polymorphonuclear ...	86	53	62	42	44
Large mononuclear ...	2	3	22	11	16
Lymphocyte	9	39	14	46	38
Large lymphocyte ...	0	2	2	0	0
Eosinophile	2	3	0	1	2
Mast	1	0	0	0	0
Crescents	16	27	12	8	20

In this case the continuance of the gametes in the peripheral blood is striking. The polymorphonuclear decrease and the lymphocytosis should be noted. This patient received no quinine during the period between November 11 and December 6. Young malignant tertian forms were always present in his blood with gametes. His temperature was irregular, and irregularly quotidian in character.

53,937: Spaniard, on the Isthmus twenty months, temperature normal, blood contained on admission many crescents but no young parasites; quinine, grains 10, *ter. die*, with Fowler's solution, gtts. 5.

CHART NO. 53937

	Feb. 5	Feb. 6	Feb. 8	Feb. 9	Feb. 11	Feb. 14	Feb. 15	Feb. 16	Feb. 17	Feb. 19
Polymorphonuclear ...	69	41	41	42	40	52	33	40	39	52·5
Large mononuclear ...	8	33	20	11	19	11	20	18	23	7·5
Lymphocytes	18	17	23	34	28	22·5	24	25	23	23
Large lymphocytes ...	3	8	10	8	5	10·5	6	14	10	7
Mast	0	0	0	0	1	0·5	0·3	0	2	0·5
Eosinophile	2	1	6	5	7	3·5	9	3	3	9·5
Crescents	92	87	61	48	20	3·5	4	3	2	0·5

This table illustrates, as in the preceding one, the steady disappearance of crescents under quinine, grains 10, *ter. die*, also the variation in the proportions of leucocytes.

It should be said that the specimens of blood were always taken at 4.30 or 8.30 p.m., or about four hours after a meal.

53,742: Spaniard, sixteen months on the Isthmus; blood: malignant tertian rings, crescents, ovoides; spleen enlarged to umbilicus. February 1, quinine, grains 10, *ter. die.*; February 6, Fowler's solution, gtt. 5, *ter. die.*

HISTORY No. 53742

	Feb. 1	Feb. 2	Feb. 3	Feb. 5	Feb. 6	Feb. 8	Feb. 9	Feb. 11
Polymorphonuclear	56	48	36	44	56	42	43	42
Large mononuclear	17	22	16	15	12	18	25	27
Lymphocytes	15	21	27	24	8	18	14	12
Large lymphocytes	8	4	3	8	10	12	8	9
Eosinophile	3	5	15	7	13	9	10	9
Mast	1	0	2	1	1	1	0	1
Pigmented leucocytes	—	—	1	1	—	—	—	—
Crescents	29	22	29	14	10	5	5	2

Notes :—Blood, February 2, 6,500 leucocytes per mm.³.
Blood, February 5, containing one phagocyted gamete.

The large mononuclear increase is striking.

M. L., Spaniard, malignant tertian malaria, returned and died. Autopsy, March 28, 1909. Quinine grains 10, *ter. die.*

	Dec. 26	Dec. 27	Dec. 28	Dec. 30	Dec. 31	Jan. 2	Jan. 13
Polymorphonuclear	48	65	60	64	70	65	45
Large mononuclear	13	6	5	9	3	12	5
Lymphocyte	34	25	32	14	22	14	34
Eosinophile	4	4	3	12	5	8	16
Mast	—	—	—	1	—	1	—
Pigmented leucocyte	1	—	—	—	—	—	—
Crescents	9	5	5	4	1	0	0

Note :—Poikilocytosis and basophilia of red blood corpuscles on January 13.

The following two records are taken from cases of simple tertian malaria receiving quinine, grains 10, *ter. die*.

History No. 51147

	Dec. 21	Dec. 23	Dec. 26
Polymorphonuclear	69	18	48
Large mononuclear	7	39	12
Lymphocyte	23	36	30
Mast	1	—	—
Eosinophile	0	7	10
Gametes	3	1	0

Quinine discontinued after an initial dose.

History No. 50792

	Dec. 14	Dec. 15	Dec. 17	Dec. 20
Polymorphonuclear	64	48	17	23·5
Mononuclear	19	17	28	24
Lymphocyte	7	15	45	41
Eosinophile	10	19	8	10
Mast	0	1	1	0·5
Pigmented leucocyte	0	0	1	0·5
Gametes	?	?	0	0

Quinine discontinued after an initial dose. Returned January 11 with malignant tertian malaria—seven crescents per hundred leucocytes.

Hospital No. 50782

	Dec. 14	Dec. 15	Dec. 17	Dec. 20	Jan. 13
Polymorphonuclear	47	40	20	47	42
Large mononuclear	34	34	39	13	11
Lymphocyte	16	26	37	37	42
Eosinophile	3	0	4	2	4
Mast	0	0	0	1	—
*Gametes	0	0	0	0	—

*None of seven *Ce. albimana* became infected from this case, indicating the absence of gametes.

In each of the above cases the rapid polymorphonuclear decrease and the equally rapid mononuclear increase should be noted.

The effect of quinine administration, then, is to make the gametes gradually disappear from the peripheral blood by the destruction of the young forms, the gametes being phagocyted by splenic and hepatic endothelium. It is concluded that quinine, grains 10, *ter. die*, in solution, will gradually reduce the sexual form of the parasite in man to a non-infective minimum in from a few days to a few weeks, depending on the severity of the infection.

In simple tertian malarial fever, gametes disappear from the peripheral blood within two or three days under quinine treatment, and generally disappear even when quinine is withheld, if the patient is at rest. There are never as many gametes in the peripheral blood in simple tertian as in malignant tertian malaria. As a consequence, one never finds as many simple tertian zygotes as malignant tertian zygotes in infected mosquitos.

PRELIMINARY EXPERIMENTS ON THE EFFECT OF COLD ON VARIOUS DISEASES IN SMALL ANIMALS

BY

PROFESSOR MAJOR RONALD ROSS, C.B., F.R.S.,

AND

MAJOR C. L. WILLIAMS, I.M.S. (RETIRED)

(*Received for publication 11 June, 1910*)

Prefatory Note by R. Ross

Some years ago Sir Edwin Durning-Lawrence, Bart., offered funds to the Liverpool School of Tropical Medicine for the purpose of experimenting on the effect of cold on Yellow Fever—a subject in which he had long been interested owing to various reports which had come to his knowledge. The School found it difficult to give effect to his wishes, and the matter was allowed to drop.

For a long time I have felt that much experimental work still remains to be done regarding the cure of parasitic diseases, especially by such simple natural agencies as heat and cold. Most of the animal parasites occur most frequently in persons who live in warm climates, that is to say, they are accustomed to live in hosts who themselves live under conditions favourable to the said parasites. There may be reasons for thinking that if these conditions are abruptly altered the result will be harmful to the parasites, just as abrupt changes are apt to be harmful to higher organisms. Of course, the temperature of the patient's body is not markedly changed by alteration of external temperature; but, nevertheless, certain changes may be produced in his blood or tissues which are likely to be inimical to parasites living in him. I cannot point to any very definite prima facie evidence in favour of this view, but some general observations may be mentioned. For example, it is a common custom to send patients suffering from malaria to temperate climates, as to the hills in India. From my own clinical experience I certainly think it is easier to treat malaria in

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England than in the tropics, and easier to treat it in the cool season in the tropics than in the very hot season—that is to say, I think that a given dose of quinine will produce least effect in the hot climates in which the parasites are most easily propagated. Moreover, if we make a careful study of statistics in India, we often observe a sudden rise in the malaria rate at the end of the cold season, long before any new brood of Anophelines has hatched out. True, this rise may be partly explained by the emergence of old Anophelines from hibernation; but it may also be due to relapses caused by the greater heat. There are also many observations on record, especially in Italy, suggesting that season affects the plasmodia, especially the sexual forms, quite apart from the proliferation of the carrying agents. Human trypanosomiasis occurs in persons in very hot climates, and may just possibly be retarded if such persons are removed to cold ones. In one case of complete cure known to me, marked improvement occurred when the patient was subjected to considerable cold, accompanied by healthy exercise and open air in Scotland. Dysentery seems to improve much more rapidly in cool climates than in hot ones. Possibly the effect may only be a slight one; but it is well worth while to study whether there is any effect at all, and if so how much—because anything that concerns so important a matter as the treatment of disease is worth considering. Still greater improvement might be effected in many maladies, especially diseases of the respiratory system, and of the skin, if cold is combined with a very dry and pure atmosphere, and, of course, with other forms of treatment.

Last year, therefore, I asked Sir Edwin Durning-Lawrence whether he would not assist us to carry out researches on these points in Liverpool. He assented at once, and suggested that I should ask his friend, Sir Alfred Haslam, the head of the great refrigerating works at Derby to construct a chamber capable of being kept at the requisite temperature. The result was that the Haslam Engineering Company made such a chamber at the University Laboratories of this School It was completed on September 10th, 1909, and after a few interruptions, has been kept continuously running ever since. Major C. L Williams was put in charge of the experiments from early in September until the 13th April, 1910, since when the work has been conducted by Dr. J. G. Thomson. At the time when Dr. J. G.

Thomson commenced his labours, Dr. David Thomson and myself had just elaborated what we call Enumerative Methods* for the study of parasitic diseases, and these have now been employed for the Cryotherapy work. In this paper, however, we record only the preliminary experiments conducted by the old methods—which were the only ones used by Major Williams.

Our warm thanks are due to Sir Edwin Durning-Lawrence for his munificence regarding this extensive line of research, and to Sir Alfred Haslam for the great interest which he has taken in the matter.

Summary of Experiments from the 10th September, 1909, to 13th April, 1910

By Major C. L. WILLIAMS, I.M.S.

The chamber is 12 feet long by 7 feet wide by $6\frac{3}{4}$ feet high, with a cubic content of about 540 feet, and can be kept at any temperature between about 15° F. (−9·4° C.) and 150° F. (65·5° C.). The motor was worked usually from about 7.45 a.m. to 5.30 p.m., but had to be stopped at intervals, perhaps for an hour or two at a time whenever 20° F. was reached. Usually it rose during the night to 36° or 38° F., or thereabouts, and that would be the initial temperature at 7.30 a.m. at the time of starting the machinery. The temperature was reduced by an ammonia compressor worked by a 6-h.p. motor and a fan driving in air through a chamber in which a saturated solution of calcium chloride was kept constantly trickling over corrugated iron plates.

In theory the air is not supposed to change or be refreshed by additions of outside air, but apart from the rush of air into the chamber each time the door was opened, a procedure necessary several times a day, the absence of smell and the practically inappreciable amount of CO_2 and organic matter in the air when tested chemically point very strongly to a rapid removal of respiration products: and, moreover, the animals in the chamber at no time showed any signs of intoxication by impurities of re-breathed air.

The low humidity of the air in the chamber is no doubt a strong factor in the results. It varied usually from about 50 to 60 per cent.,

* Vide page 261 of this number.

TABLE SHEWING THE COURSE OF VARIOUS TRYPANOSOMIASES UNDER ORDINARY AND UNDER COLD CHAMBER CONDITIONS UP TO FEBRUARY 21, 1910

Trypanosome	Animal infected	Number of animals	Incubation period in days			Period lived in days			Failed to shew infection	Remarks
			Longest	Shortest	Average	Longest	Shortest	Average		
T. lewisi ...	Rat ...	13 (Cold chamber 6)	25	4	14	127 (Still alive)	20	40	2	One still alive (21.2.10) shewed trypanosomes for 35 days. Its weight has risen from 85 to 163 grams in 127 days (its coat, however, is much longer, and it was probably young when received). Two died after 45 and 53 days, of epidemic pneumonia.
		(Controls 7)	12	4	8	112 (Still alive)	21	46	3	Two died after 12 and 69 days respectively of epidemic pneumonia. One is still alive.
T. equiperdum...	Rat ...	28 (Cold chamber 14)	20	6	13·4	36	12	25	1	Note that the sole failure was in the cold chamber (? aborted infection). One died soon after inoculation.
		(Controls 14)	24	8	17·8	38	21	26·8	—	
T. brucei ...	Guinea-pig	15 (Cold chamber 8)	36	4	18	62	20	37·1	—	One died soon after inoculation.
		(Controls 7)	35	5	17·1	57	20	34·1	—	
T. evansi ...	Guinea-pig	6 (Cold chamber 3)	21	18	19·5	26	24	25	1	
		(Controls 3)	10	Not known	0	21	Not known	21	1	One died in five days (? cause), before any trypanosomes had shewn in the peripheral blood.

NOTE.—Some of these animals were removed for ten days to the animal house whilst the cold chamber was under repair, and for that amount of time were subject only to ordinary temperatures.

NOTE.—One 'lewisi' rat is still alive (12.4.10) in the cold chamber, and seems lively and well. It has been in 177 days, and still shews occasional trypanosomes.

being higher on days when the humidity outside was high, and being raised each time the door had to be opened to admit of feeding, taking observations, etc. To the observer it proved very invigorating, comparable probably to the air in the interior of Canada. The animals in the chamber, too, seemed more active and more interested in their surroundings than the controls which were kept in a greenhouse, artificially heated, but with a very varying temperature and with the humidity of the outside air. Curiously enough, the animals in the control house threw out actually longer coats than those in the cold chamber, and their appetites were markedly less.

The animals subjected to the influence of the cold were guinea-pigs, rats and mice. The animals were placed in cages, well bedded and well fed, fat and proteid especially being provided in their diet.

The diseases brought under the influence of the cold were chiefly various trypanosomiases, with, in addition, tubercle (bovine), cancer (in mice), tetanus, and spirochaetosis, and the results during over eight months' observation are tabulated below. During the eight months there were occasional intermissions for a few days for repairs to machinery, etc., but they amounted to but quite a few days in all.

In the table it will be noted that four animals died of pneumonia, an epidemic of which, unfortunately, occurred at one period and carried off several of the animals under observation. It was probably introduced with some wild rats placed at first near the controls, but affected both controls and cold chamber subjects equally.

The influence of *T. lewisi* is, in any case, difficult to ascertain as rats recover spontaneously from this affection; but it will be noted that the only animal which failed to show trypanosomes after inoculation with *T. brucei* was one in the cold chamber. The dose in all cases was, of course, the same, in terms of c.c. for the animal observed and its control, but the actual number of trypanosomes injected must vary within very wide limits, and this factor will influence the incubation periods. Whereas it seems to be somewhat delayed in *T. lewisi* it varies but little in the other trypanosomes. There was a small prolongation of life in the Ngana and Caderas cases, but this did not hold good for *T. lewisi* and Dourine.

Twelve mice were inoculated with cancer, and six put into the cold chamber; five lived 50, 51, 56, 70 and 90 days respectively, whereas of their six controls, two lived 56 and 76 days respectively.

Of the remainder, three controls failed to develop tumours, and one each in the control and cold chambers died within a few days of inoculation.

Six mice were also put into the cold chamber uninoculated as controls, and of them two are still alive (21 Feb.), well and vigorous, after 120 days, showing that cold *per se* had no bad effect on them; whilst two were eaten by their comrades after respectively 24 and 42 days' residence in the cold chamber, and two died of natural causes in 56 and 62 days.

Some mice were also infected with *Spirochaeta duttoni*, but no appreciable influence on the course of this disease could be observed in them.

Five guinea-pigs with tetanus were placed under observation, a case of a boy cured of that disease whilst being treated in a refrigerator having been reported by Crane ('St. Louis Medical Review,' 7th July, 1906). Of the tetanus guinea-pigs, one injected with $\frac{1}{500}$ c.c. of a culture and placed in the cold chamber, died in $9\frac{1}{2}$ days, but it is very doubtful if it died of tetanus; two injected with $\frac{1}{1500}$ and $\frac{1}{1000}$ c.c. respectively and kept as controls never showed any symptoms; whilst two injected with $\frac{1}{500}$ c.c. and placed one in the cold chamber and one as control died with similar symptoms in a similar time—something over 50 hours.

An Englishman suffering from sleeping sickness of some three months' duration, and contracted in N. Rhodesia, was submitted to a course of treatment in the chamber from January 5 onwards to the end of that month. Ordinarily he came from the hospital in which he was by cab about 10 a.m., and rested in the chamber, well wrapped up, till about 3.30 p.m. In all he had a course (interrupted for some days) of twelve days, or some 53 hours in all, of cold air. The temperature was gradually decreased till 24° F. was reached. He himself said he felt much better for the treatment, which acted on him as a tonic; but in the short period he was under observation no visible diminution was apparent in the number of trypanosomes in the blood. He was getting atoxyl simultaneously at his hospital. Several times he walked back—a distance of fully $1\frac{1}{2}$ miles—after his cold chamber rest; but since February 1 he has not been able to leave the Hospital, and has therefore been unable to utilise the action of cold in altering his metabolism, and thereby the culture media within his body of *T. gambiense*.

As regards increase or loss of weight in the animals under observation, most of them were young, and their weights, of course, increased by mere growth. Heavier coats also added to the weight. Of those on which accurate data can be founded the results are as follow:— Number of animals: Rats, 19; guinea-pigs, 8. Rats: Gained weight, 14; lost weight, 5. Guinea-pigs: Gained weight, 6; lost weight, 2.

Animal	Cold Chamber		Controls		Total
	Gained weight	Lost weight	Gained weight	Lost weight	
T. lewisi (rats)	3	1	1	1	6
T. equiperdum (rats)	6	1	4	2	13
T. brucei (guinea-pigs)	3	—	2	—	5
Tubercle	—	1	1	—	2
T. evansi	—	—	—	1	1
Total	12	3	8	4	27

Put shortly, all the animals in the cold chamber gained weight except three, of which one was a case of tubercle: showing, on the whole, and as far as the small figures go, a favourable influence on their general nutrition. But these figures are amply supported by the obvious increase in weight of practically all the animals in the chamber, though their immaturity in most cases makes the actual results in grams of weight gained of less value from the point of view of influence of cold *per se.*

Finally, as regards the temperatures of the animals, no constant effect could be observed in the influence of the cold chamber, and those in and those out varied equally irregularly, with no permanent ratio between the one and the other.

Note on 12 April, 1910. The result of further experiments carried out since February 21 and bringing the observation period up to over eight months is as follows:—

Six guinea-pigs were inoculated with bovine tubercle (0·1 mgr. culture) on the 27th November, and three placed in the cold chamber

and three outside, with, of course, uninoculated controls in both cases. The uninoculated controls throve and call for no remark, except that one in the cold chamber died after 34 days of no obvious cause, there being only a little lung congestion evident in post mortem. Of the inoculated, all are still alive, and those in the cold chamber have now been in it 137 days, and seem quite fit and well. One female has aborted twice, but whether from the cold, the tubercle, or other cause, cannot be definitely stated. Only three were apparently mature at the time of inoculation, and their weights in the case of two inoculated have risen in one case from 400 to 653 grams, and fallen in the other from 772 to 747; whilst the third, uninoculated, has risen from 622 to 794. Their temperatures have not shown any very striking variations; but the swelling over the site of inoculation burst fully a month sooner in the two controls outside, in which rupture has occurred, than in the one in the cold chamber, which developed suppuration; and this, as far as it goes, points to some diminution of the virulence of the bacilli in the cold chamber.

Four guinea-pigs have since (22 March) been inoculated with human tubercle, and three of them are at present under observation, one having died soon after inoculation.

Six guinea-pigs were inoculated with *T. gambiense* on the 14th December, and, as usual, placed three in the cold chamber and three outside as controls. All failed to show trypanosomes in the peripheral blood. One in the cold chamber died 16 days after inoculation, but no trypanosomes were found in the heart's blood post mortem, and it probably died of other causes. The other five were again inoculated on the 4th March, and again failed to show trypanosomes. But a third inoculation on March 30 has been successful and interesting, inasmuch as, whereas the three outside showed parasites in their peripheral bloods on the fifth day, of the two inside one only showed them very scantily on the eighth day, and has not shown them since, the other has so far not shown them at all: a result pointing strongly towards at least a delayed development in those in the colder air.

Several rats have been inoculated with cultures of pneumococcus, but so far no marked difference has been observable in their symptoms, whether in or out of the chamber, and none have died so that they remain under observation, and the final result will only become apparent later.

MALARIA PREVENTION IN JAMAICA

BY

SIR RUBERT BOYCE, F.R.S.,

DEAN OF THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

(*Received for publication 16 June, 1910*)

PLATES XVI-XVII

Although it is too early to sum up the results of the special Commission appointed in October to enquire into the malarial problem in Jamaica, nevertheless, as the methods of procedure of the Commission are of real and practical interest at a time when it is incumbent upon all colonies to take steps to abate the malaria nuisance, I have considered the narration of the following facts not without interest, as I have had myself an opportunity of examining the work in progress, and as an important paper dealing with the subject of malaria in Jamaica has appeared in a previous number of these 'Annals.'*

Appointment of the Commission

The Commission was appointed by Sir Sydney Olivier, K.C.M.G., the Governor, on October 16, 1909, and consisted of the following representative members:—

The Colonial Secretary, P. C. Cork,
The Archbishop of the West Indies,
The Senior Medical Officer, Dr. Kerr,
Dr. Turton,
Dr. Grabham, Entomologist,
Messrs. Gideon and Hurt.

Mr. F. N. Isaacs was subsequently appointed Secretary. The selection of the Commission was a wise one, the members being fully alive to the importance of the work—sanitarians of pronounced modern views, and in several instances gentlemen who had contributed valuable papers upon the subject, or who had had previous experience of anti-malarial work in other colonies.

* Annals of Trop. Med. and Parasit. Vol. III, No. 4.

Power and scope of the Commission

The Commission had considerable power and a comparatively free hand. They were directed to conduct investigations, to summon witnesses if necessary, employ inspectors and experts to assist and advise them. They were, furthermore, empowered to carry out remedial and preventive works for the abatement of malaria independent of local sanitary bodies, and to conduct medical and biological investigations and to report from time to time. They were authorised to spend for scientific and preventive measures a sum in the first instance not exceeding £500 without obtaining further authority. They were also asked to report upon, and to devise measures to prevent other diseases which they might come across in the course of their investigations.

First steps of the Commission

This consisted in ascertaining the prevalence and distribution of malaria in the Island. In the first place the Commission paid a just tribute to the Report upon Malaria in Jamaica drawn up by Dr. Prout,* in which both the causes of malaria and the principles necessary for its control were fully discussed.

In the second place they asked for returns of all the cases of malaria in the island, and Dr. Grabham, one of the members of the Commission, took a splenic census of the West end district of Kingston. The Secretary, Mr. Isaacs, proceeded to Annotto Bay to make a survey of the town and district in order to ascertain the anti-malarial measures which could with advantage be introduced. Similarly the Black River, Yallahs, Morant Bay and Bath districts were visited and inspected for breeding places of Anophelines, in order to devise methods for their reduction.

From these preliminary investigations the Commissioners were able to report in February, 1910, that one-half the Island was practically free from malaria-carrying mosquitos. This area comprised those parts of the Island above 1,000 feet. Below 1,000 feet the Anophelines were found in varying numbers, and were greatest in the low-lying plains near the sea coast, where natural drainage was most difficult. Breeding places existed in the East and West end districts of Kingston and the West End constituted a badly

* *Loc. cit.*

infected endemic centre; a spleńic census taken by Dr. Grabham in December, 1909, disclosing a malaria rate of 65 per cent. The cases which were received into the Kingston Hospital came mainly from this district. In this district also investigations disclosed the interesting fact that the breeding places were to a very large extent artificial, and therefore preventable collections of water, mainly due to the abuse of the filtered water supply of the town and to neglected drains and gullies. In company with Mr. Isaacs, I visited this district in April of this year—1910—and observed for myself a miniature but complete system of irrigation consisting of streams, rills, ponds and pools, all fed and kept up by the simple device of turning the taps of the domestic water supply full on, night and day. This method yielded an abundant supply of water, and enabled an extensive and profitable market-gardening system to be carried on in the midst of Kingston. Looking down upon the town, the district in question could be readily picked out by reason of its conspicuous green appearance, the result of the growth of banana trees and vegetation generally. The houses of the coolies and others who were the principal cultivators in the district were very numerous, and in these the Anophelines kept up an abundant supply of malaria cases, and helped to spread the disease to wider areas around. Thus Kingston presented a picture, of on the whole, a well-planned and drained town, from which yellow fever and malaria had been driven out and were no longer endemic; but in it there existed a comparatively small, thickly-populated area where malaria was still endemic, and the cause of the formidable number of cases of malaria entered on the books of the Public Hospital. A more striking and instructive picture of the inter-relationship of Anophelines and their breeding places with the prevalence of malaria could probably not be easily paralleled in a similar area anywhere. It serves to conclusively demonstrate the utility and importance of making investigations. The unnecessary suffering and expenditure of money caused by this endemic focus were naturally considerable, and the wanton waste of money caused by utilising the *filtered* town water supply for market-gardening purposes has a comical aspect were it not associated with such serious consequences. The Commissioners were not slow in pointing out how readily this glaring abuse might be remedied, and how by the construction of better drains and by

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First steps of the Commission

This consisted in ascertaining the prevalence and distribution of malaria in the Island. In the first place the Commission paid a just tribute to the Report upon Malaria in Jamaica drawn up by Dr. Prout,* in which both the causes of malaria and the principles necessary for its control were fully discussed.

In the second place they asked for returns of all the cases of malaria in the island, and Dr. Grabham, one of the members of the Commission, took a splenic census of the West end district of Kingston. The Secretary, Mr. Isaacs, proceeded to Annotto Bay to make a survey of the town and district in order to ascertain the anti-malarial measures which could with advantage be introduced. Similarly the Black River, Yallahs, Morant Bay and Bath districts were visited and inspected for breeding places of Anophelines, in order to devise methods for their reduction.

From these preliminary investigations the Commissioners were able to report in February, 1910, that one-half the Island was practically free from malaria-carrying mosquitos. This area comprised those parts of the Island above 1,000 feet. Below 1,000 feet the Anophelines were found in varying numbers, and were greatest in the low-lying plains near the sea coast, where natural drainage was most difficult. Breeding places existed in the East and West end districts of Kingston and the West End constituted a badly

* *Loc. cit.*

infected endemic centre; a spleńic census taken by Dr. Grabham in December, 1909, disclosing a malaria rate of 65 per cent. The cases which were received into the Kingston Hospital came mainly from this district. In this district also investigations disclosed the interesting fact that the breeding places were to a very large extent artificial, and therefore preventable collections of water, mainly due to the abuse of the filtered water supply of the town and to neglected drains and gullies. In company with Mr. Isaacs, I visited this district in April of this year—1910—and observed for myself a miniature but complete system of irrigation consisting of streams, rills, ponds and pools, all fed and kept up by the simple device of turning the taps of the domestic water supply full on, night and day. This method yielded an abundant supply of water, and enabled an extensive and profitable market-gardening system to be carried on in the midst of Kingston. Looking down upon the town, the district in question could be readily picked out by reason of its conspicuous green appearance, the result of the growth of banana trees and vegetation generally. The houśes of the coolies and others who were the principal cultivators in the district were very numerous, and in these the Anophelines kept up an abundant supply of malaria cases, and helped to spread the disease to wider areas around. Thus Kingston presented a picture, of on the whole, a well-planned and drained town, from which yellow fever and malaria had been driven out and were no longer endemic; but in it there existed a comparatively small, thickly-populated area where malaria was still endemic, and the cause of the formidable number of cases of malaria entered on the books of the Public Hospital. A more striking and instructive picture of the inter-relationship of Anophelines and their breeding places with the prevalence of malaria could probably not be easily paralleled in a similar area anywhere. It serves to conclusively demonstrate the utility and importance of making investigations. The unnecessary suffering and expenditure of money caused by this endemic focus were naturally considerable, and the wanton waste of money caused by utilising the *filtered* town water supply for market-gardening purposes has a comical aspect were it not associated with such serious consequences. The Commissioners were not slow in pointing out how readily this glaring abuse might be remedied, and how by the construction of better drains and by

more vigorous filling-in operations this endemic focus might be rendered malaria-free. The photographs which I have had taken, and which are inserted in this paper, show very conclusively the extent of Anopheline breeding grounds which the abuse of the domestic water supply can bring about, if not properly controlled.

The Commission started filling-in operations in the offending district, and took steps to distribute quinine at cost price to the authorities of all malarial districts. They also arranged for the training of sanitary inspectors, so that the latter could be in a position to ferret out breeding places; Dr. Grabham took on himself this part of the work of the Commission. Public lectures were also delivered at various centres throughout the Island. In conclusion, the Commission has in a short space of time done good work—it has ventilated the subject of malaria; it has placed its finger upon the offending spots; it has shown with what comparative ease and at what small cost the remedy can be obtained; and it has commenced to enforce the remedy.

Tables showing the deaths from malaria in the various parishes from the year 1897 are appended.

DEATHS FROM MALARIA

Parish	1897-98	1898-99	1899-1900	1900-01	1901-02	1902-03	1903-04	1904-05	1905-06	1906-07	1907-08	1908-09
Kingston	68	46	50	31	71	50	42	47	88	89	89	54†
St. Andrew	18	15	20	15	25	27	16	18	26	18	37	10
St. Thomas	3	7	6	6	9	33	41	17	35	45	72	57†
Portland	34	46	85	40	39	71	124	100	77	87	112	105†
St. Mary	2	4	15	7	5	34	72	40	35	36	49	22
St. Ann	6	3	7	4	1	11	10	8	7	10	20	18
Trelawny	18	5	2	9	1	5	3	10	10	4	12	7
St. James	10	9	5	7	12	12	12	14	18	12	14	12
Hanover	2	1	—	2	1	4	1	—	3	2	1	4
Westmoreland ...	10	3	1	3	4	28	20	22	20	38	28	13
St. Elizabeth	3	4	3	5	7	10	10	9	15	23	23	25†
Manchester	1	1	—	3	—	4	7	2	4	10	14	7
Clarendon	14	7	11	7	14	38	31	36	33	43	34	23
St. Catherine ...	8	10	6	2	12	41	80	84	90	94	165	109†
Whole Island ...	197	161	211	141	201	368	469	407	461	512	670	466

Death Rates for 'Malaria' for the years 1897-98 to 1908-1909

Year	Malaria			
	Rate per 100 deaths		Rate per 1000 of population	
	Parish of Kingston	Whole Island excluding Kingston	Parish of Kingston	Whole Island excluding Kingston
1897-1898	4·5	0·8	1·3	0·1
1898-1899	3·2	0·8	0·8	0·1
1899-1900	3·4	1·0	0·9	0·7
1900-1901	2·1	0·7	0·5	0·1
1901-1902	4·8	0·8	1·3	0·1
1902-1903	3·6	2·2	0·9	0·4
1903-1904	2·6	2·3	0·7	0·5
1904-1905	2·9	1·9	0·8	0·4
1905-1906	5·6	2·2	1·6	0·4
1906-1907	4·3	2·1	1·6	0·5
1907-1908	4·5	2·6	1·6	0·7
1908-1909	2·9	2·4	0·9	0·5

Plates XVI-XVII

Anopheline Breeding-pools in Jamaica

P. P. Press, imp.

P. P. Press, imp.

ON SOME SPECIES OF *CYCLOPS* AND OTHER ENTOMOSTRACA COLLECTED BY DR. J. M. DALZIEL IN NORTHERN NIGERIA

BY

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(*Received for publication 21 June, 1910*)

PLATES XVIII-XX

The species here described were collected by Dr. Dalziel at Yola in Northern Nigeria, in the course of a research on the Life History of the Guinea Worm. The specimens were sent to me for description by Mr. J. H. Ashworth, of the Zoological Department of Edinburgh University, and examples, as far as possible, of the various species have been sent to the University for future reference. Some few types are, however, unavoidably absent. Dr. Dalziel made careful notes of the various localities from which his specimens were taken, which I here transcribe. The sources of each particular species are indicated by the letters affixed to the descriptions.

Sources.

A. Turbid bush-pools with muddy bottom, frequented by cattle and containing fish.

B. Surface-well in clay, free from water plants, but with grass and weeds dipping down the sides to the water's edge.

C. Marshes or pools of clear water when undisturbed; bottom of mud or of grass coated with sediment; containing *Lemna*, *Nymphoea* and other water plants, and fringed with rank grass.

D. Small pools of clear water in shrinking bed of River Benué, recently isolated and therefore not long stagnant, and containing flocculent viscid algae, insect larvae, etc.

E. Benué River, small bays and backwaters of clear water but no current, sandy edges and bottom with some vegetable débris and sediment, but no growing vegetation.

It may be useful to future workers in this field to note here some of the points which need special attention in determining the various species of *Cyclops*.

1. The number of joints in the anterior antennae and the length of the limb relatively to the body of the animal.

2. The numbers of joints in the rami of the four pairs of natatory feet.

3. The characters of the rudimentary fifth pair of feet.

4. The characters and proportional length of the caudal rami.

The general outline of the body and its various segments should be noted, and in living specimens the colours of the body and egg-sacs may provide useful characters.

Dr. Dalziel's attention seems to have been almost entirely directed to the Cyclopidae as being probably the intermediate bearers of human parasites, but it is quite likely that these hosts might also be found among the Ostracoda. In two, at least, of the British species of that group I have myself found scolices of an undetermined species of Taenia, and in yet another Ostracod many specimens of a larval Trematode worm, as well as a fully developed worm belonging to the group Acanthocephala. A brief reference to these may be found in my paper on the British species of Candoninae. (Proceedings of the Zoological Society of London, 1910, Part 1.)

COPEPODA

Clycops nigeriae, n. sp. Plate XVIII, figs. 1-7.

Female, length 0·88 mm. Body robust (fig. 1), cephalic segment as broad as long, rounded and slightly produced in front; the two following segments expanded laterally and obtusely angulated behind; last thoracic segment very small; urosome rather short and stout, about one-third as long as the anterior portion of the body; genital segment moderately dilated; caudal rami as long as the united lengths of the last two segments, slightly tapering distally, seta of the outer margin attached rather behind the middle, apical setae long, the innermost considerably longer than the entire urosome (fig. 3). Anterior antennae (fig. 4) eleven-jointed, reaching when reflexed to the middle of the second body-segment, rather sparingly clothed with setae of moderate length. Natatory feet short and

stout, with both rami bi-articulate (fig. 6); last pair of feet two-jointed (fig. 2), the basal joint short and not very distinct, apical joint slender, bearing two long setae, the distal one being needle-shaped. Colour stated by Dr. Dalziel to be 'greyish green, eye, deep red.' The general characters of this species are very similar to those of *C. gracilis*, Lilljiborg, and *C. bicolor*, G. O. Sars, and the natatory feet are not unlike those of *C. pachycomus*, one of the many species described by the latter author from Lake Tanganyika.

Dr. Dalziel's specimens are from several different sources. The species would seem to be generally distributed in the region investigated by him. Sources A. B. C. D. E.

Cyclops virescens, G. S. Brady. Plate XVIII, figs. 8-16.

Female, length 0·65 mm. Body slender, the anterior segment ovoid in form, and scarcely at all produced in front (fig. 8), the other thoracic segments not expanded laterally, last segment short (fig. 10); urosome slender, genital segment equal in length to the two following segments, scarcely at all dilated (fig. 10), caudal rami short, about equal in length to the last tail segment, not at all divergent. Anterior antennae (fig. 9), eleven-jointed, reaching when reflexed to the posterior border of the third body segment, slender and sparingly setiferous; natatory feet (figs. 11, 15, 16), with both branches three-jointed; fifth pair of feet uni-articulate and bearing two apical setae (fig. 14). First segment of the urosome in the *male* produced ventrally into a slender spine (fig. 13).

This, like the preceding species, occurred in various gatherings, and Dr. Dalziel's notes assign to it a wide range of colour, from yellow to green and brown. Sources A. B. C. D. E.

There can, I think, be little doubt as to identifying Dr. Dalziel's specimens with those already imperfectly described by me from Dr. Graham's collection under the specific name of *virescens*. Dr. Graham's notes, made from an examination of freshly gathered specimens, assign ten joints to the anterior antennae, whereas the number of joints in Dr. Dalziel's specimens is, so far as I can make out, eleven. But the jointing, as seen in spirit-specimens, is very indistinct, and in any case the slight difference might possibly depend upon the age of the animals. In all other respects the two forms agree with each other.

I am indebted to Dr. Calman, of the British Museum, for the oppor-

tunity of examining the type specimens of *C. virescens*, and it is on this that the foregoing remarks are based.

Cyclops longistylis ?, G. S. Brady. Plate XIX, figs. 17-23.

Cyclops longistylis, Brady. Notes on Dr. Graham's collection of Cyclopidae. (Annals of Tropical Medicine and Parasitology, Vol. 1, No. 3, 1907.)

Female, length 0·77 mm. Body slender, tapering gradually from before backwards (fig. 17), first segment longer than broad, sub-ovoid, the three following segments scarcely at all produced laterally, last thoracic segment very small; urosome slender, genital segment not at all tumid, caudal rami very slender, about six times as long as broad, scarcely divergent; anterior antennae ten-jointed, when reflexed somewhat shorter than the first body segment (fig. 22). All the natatory feet have both branches three-jointed; feet of the fifth pair very minute, almost obsolete, bi-articulate (fig. 23), the first joint bearing two setae, the distal joint one seta; attached to this pedigerous segment just in front of the foot are three rather strong setae—longer than the foot itself. The spines of the second and third pairs of feet are in the *male* unusually strong (fig. 19), those of the fourth pair not quite so strong, and those of the first pair almost normal (fig. 20).

The specimens here described were found in Dr. Dalziel's gatherings from the Benué River (Source E), 'colour dark green or greenish-yellow, the egg masses brown, carried close to the body.'

The reference of these to *C. longistylis* is somewhat doubtful, and the type-specimens being now in the British Museum—very few in number, and in an imperfect condition—I am unable to verify the reference. The principal doubt arises from a discrepancy in the recognisable number of joints in the anterior antennae, Dr. Graham's record of twelve joints disagreeing with my own observation of ten joints only in Dr. Dalziel's specimens. But these details are not easily observed in spirit-specimens, and need not be absolutely insisted on.

Cyclops leuckarti, Claus. Sources A. B. C. D. E.

Cyclops bicolor, G. O. Sars. Sources A. C.

Cyclops brevipes, n. sp. Plate XX, figs. 31-34.

Female, length 0·55 mm. Body slender (fig. 31). the first segment occupying not much less than half of its entire length, rounded

and somewhat narrowed in front, the hinder segments not expanded laterally; urosome slender (fig. 33), the genital segment not dilated and scarcely longer than the next following segment, the first two segments armed with spine-like setae on their distal outer angles, last segment having its distal margin finely aculeate; caudal rami rather longer than the last abdominal segment; setae of the outer margin attached near the middle; apical setae of moderate length. Anterior antennae (fig. 34) eight-jointed, barely half as long as the first segment of the body, sparingly beset with short setae. Natatory feet short, having both rami two-jointed (fig. 34); the place of the fifth pair occupied by two short setae.

This species occurred in the same localities as those recorded for *C. nigeriae*. It is the smallest which has ever come under my notice. The characters of the urosome and anterior antennae sufficiently distinguish it from any other form.

Diaptomus nigerianus, n. sp. Plate XIX, figs. 24-30.

Female, length 1·1 mm. Anterior division of the body rather slender, of nearly equal width throughout, the posterior segment somewhat produced laterally and sharply angulated (fig. 28); urosome (fig. 25) slender, the genital segment not at all protuberant in front, the last two segments together scarcely half as long as the genital segment, and very imperfectly separated from each other; caudal rami dilated distally, scarcely as long as the two anterior coalescent segments, terminal setae broad, sub-spathulate and rather densely plumose, about twice as long as the rami themselves (fig. 25). Anterior antennae slender, scarcely exceeding the entire length of the body; posterior maxillipeds of the usual form, the distal joint exceedingly small (fig. 26); inner ramus of the last pair of legs (fig. 27) simple, much shorter than the first joint of the outer ramus; claw of the outer ramus stout, non-ciliated, terminal joint indistinct.

Male, last segment of the cephalothorax rounded off posteriorly; abdomen narrow, five-jointed; penultimate joint of the right anterior antenna bearing an apical spine, which is shorter than the next following joint (fig. 24); outer branch of the last pair of legs (fig. 30) of the right side simple, the last joint bearing an apical simply-curved claw, and on the outer margin a small papilla and a slender curved spine; the basal joint of the foot of the left side is

produced internally into a short digitiform process or rudimentary inner branch; the outer ramus short, bi-articulate, the last joint bearing a small setiferous papilla. The protopodite bears on its distal margin a couple of papilliform processes which overlap slightly the basal joint of the limb. The tail setae of the male are much more slender than those of the opposite sex, and are non-plumose.

This species seems to be more nearly allied to *D. galeboides*, G. O. Sars, than to any other described species, though whether that form be really specifically distinct from *D. galebi* of Mràzek may perhaps be doubted. The types of *D. galebi* were taken in Egypt, those of *D. galeboides* in Lake Tanganyika.

Dr. Dalziel's specimens, here described, are from 'a rocky pool of a hill-stream, 7. II. 09.'

OSTRACODA

Cypris subovata, n. sp. Plate XX, figs. 35-39.

Shell subovate, tumid; seen from the side subreniform, highest in the middle, height more than equal to half the length, extremities well rounded, the anterior being the narrower of the two, dorsal margin gently arched, ventral slightly sinuated in the middle (fig. 35); seen dorsally the outline is ovate, tumid, greatest width behind the middle and equal to considerably more than half the length (fig. 36), extremities obtusely pointed, the left valve larger than the right and distinctly overlapping in front; shell surface smooth, clothed at the extremities with fine hairs. Length 0·77 mm. Setae of the posterior antennae reaching beyond the apices of the terminal claws. Caudal rami slender (fig. 37), their terminal claws simple, only slightly curved, the marginal seta short, attached near the distal end of the limb about one-fourth of its length from the apex. Sources A. D.

Cypridopsis circinata, n. sp. Plate XX, figs. 40, 41.

Shell very tumid, sub-spherical; seen from the side sub-ovate, gibbous, greatest height situated in the middle, and equal to about three-fourths of the length, extremities very broadly rounded, dorsal margin boldly arched, gibbous in the middle, ventral slightly convex

(fig. 41); seen from above broadly elliptical, widest in the middle, width equal to two-thirds of the length, extremities evenly rounded off, lateral margins boldly and evenly arched, right valve slightly larger than the left (fig. 40). Caudal rami very small; only imperfectly seen. Shell-surface smooth, without sculpture of any kind, but densely clothed with short hairs. Length 0·65 mm. Dr. Dalziel's collection contained only one example of this species, taken in one of the two localities given for *Cypris subovata*.

CLADOCERA

Simocephalus, sp.

Diaphanosoma leuchtenbergianum? S. Fischer. Both of these from Sources A. B. C. D.

EXPLANATION OF PLATES

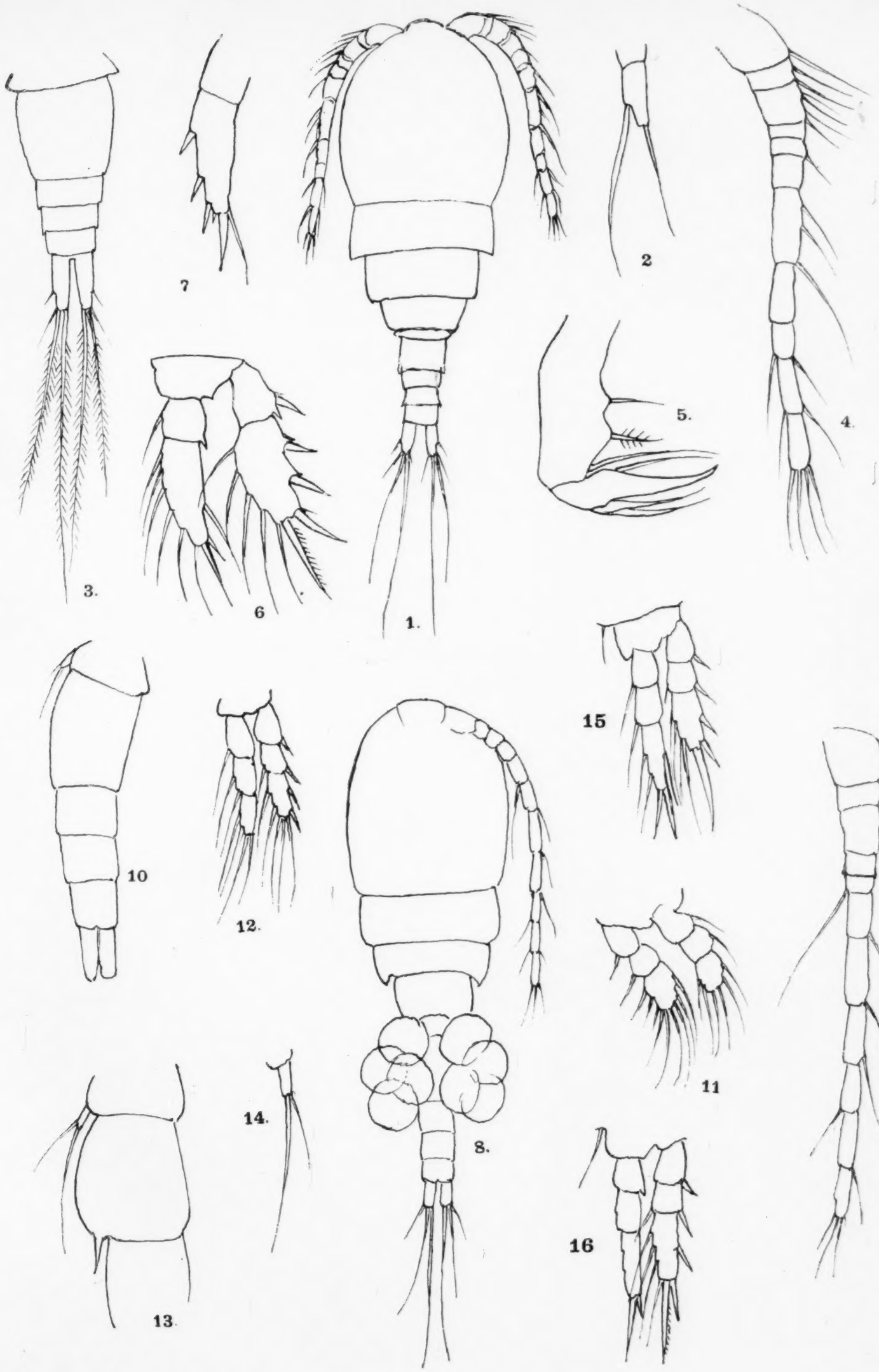
PLATE XVIII

Cyclops nigeriae ♀

Fig. 1. Female seen dorsally. × 84.
Fig. 2. Foot of fifth pair. × 440.
Fig. 3. Urosome. × 160.
Fig. 4. Anterior antenna. × 160.
Fig. 5. Posterior footjaw. × 240.
Fig. 6. One of the swimming feet. × 180.
Fig. 7. Outer branch of third pair (?). × 180.

Cyclops virescens ♀

Fig. 8. Female seen ventrally. × 120.
Fig. 9. Anterior antenna. × 240.
Fig. 10. Urosome and last thoracic segment. × 240.
Fig. 11. Foot of first pair. × 320.
Fig. 12, 15, 16. Foot of second, third and fourth pairs. × 240.
Fig. 14. Foot of fifth pair. × 440.
Fig. 13. Last thoracic segment and first segment of urosome of male. × 240.



G. S. BRADY *del ad nat.*

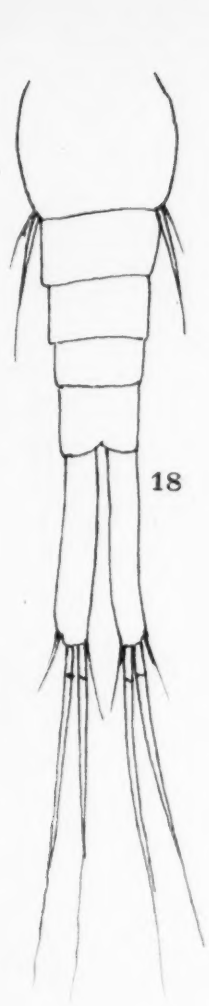
Plate XIX

Cyclops longistylis ?

Fig. 17. Female seen dorsally. × 84.
Fig. 18. Urosome. × 180.
Fig. 19. Foot of fourth pair, male. × 240.
Fig. 20. Foot of first pair. × 240.
Fig. 21. Posterior antenna. × 240.
Fig. 22. Anterior antenna. × 240.
Fig. 23. Last thoracic segment and fifth pair of feet. × 300.

Diaptomus nigerianus

Fig. 24. Part of anterior antenna of male. × 140.
Fig. 25. Urosome of female. × 140.
Fig. 26. Posterior maxilliped. × 140.
Fig. 27. Foot of fifth pair, female. × 180.
Fig. 28. Last thoracic segment, female. × 140.
Fig. 29. Female seen laterally. × 65.
Fig. 30. Fifth pair of feet of male. × 140.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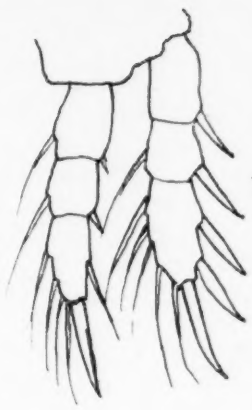
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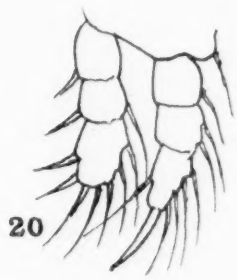
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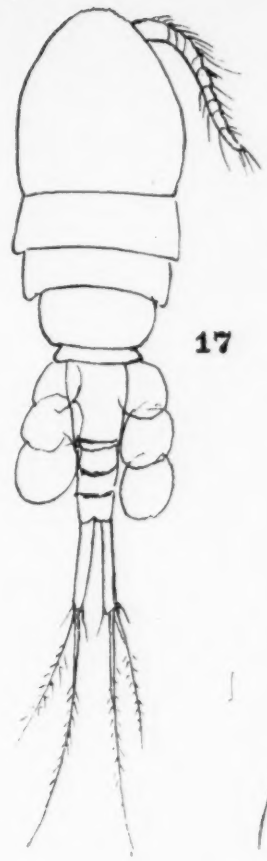
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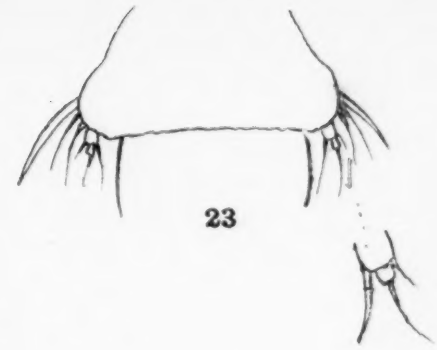
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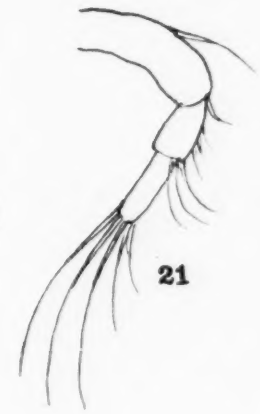
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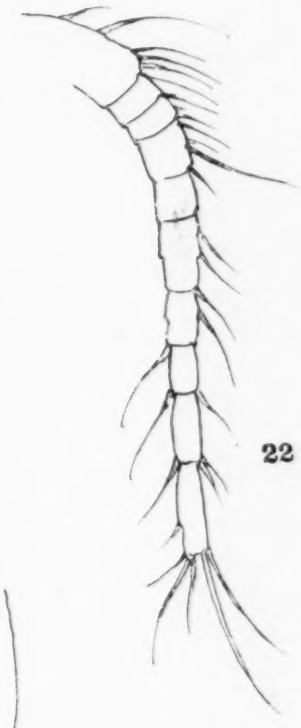
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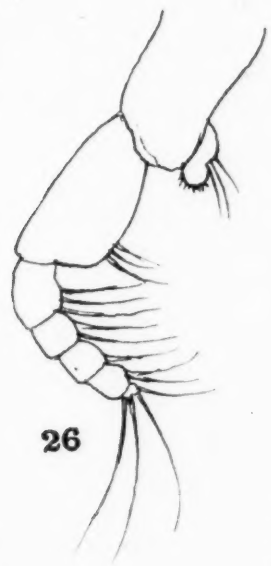
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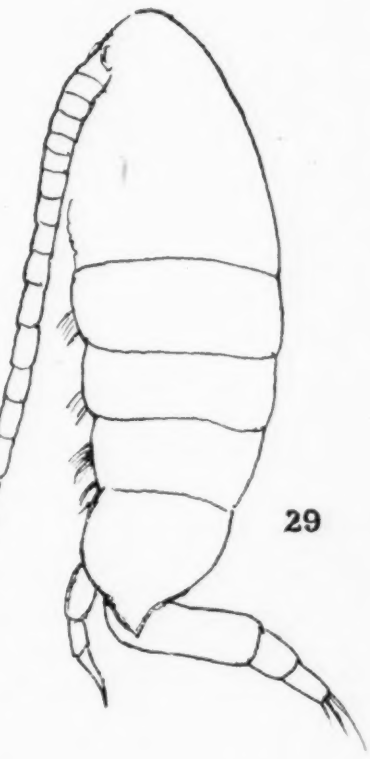
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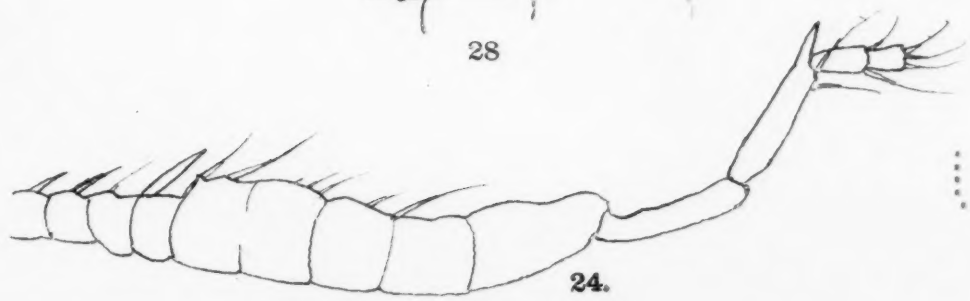
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G. S. BRADY *del ad nat.*

Plate XX

Cyclops brevipes ♀

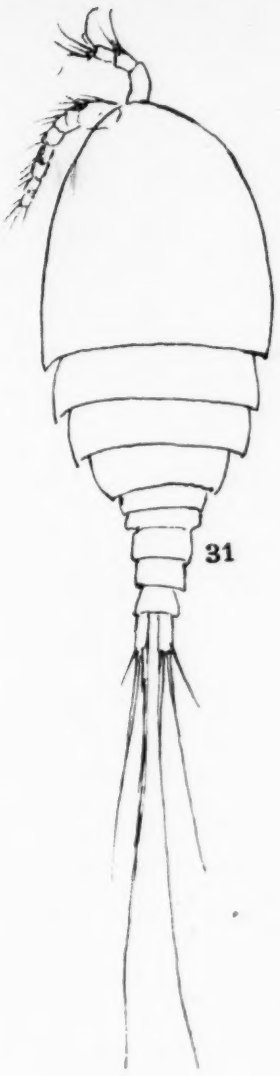
Fig. 31. Female seen dorsally. × 100.
Fig. 32. Foot of first pair. × 350.
Fig. 33. Urosome. × 300.
Fig. 34. Anterior antenna. × 350.

Cypris subovata

Fig. 35. Shell seen from left side. × 65.
Fig. 36. Shell seen from below. × 65.
Fig. 37. Caudal ramus. × 150.
Fig. 38. Extremity of foot of last pair. × 150
Fig. 39. Posterior maxilla. × 240.

Cypridopsis circinata

Fig. 40. Shell seen dorsally. × 84.
Fig. 41. Shell seen from right side. × 84.



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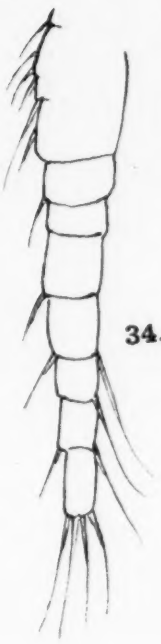

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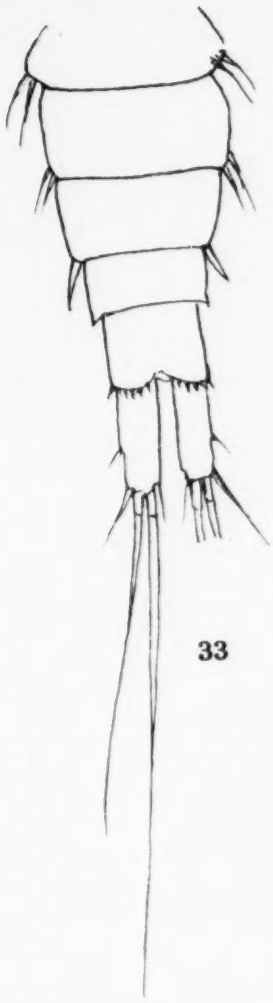

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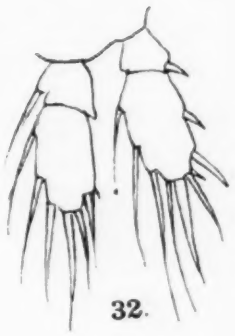

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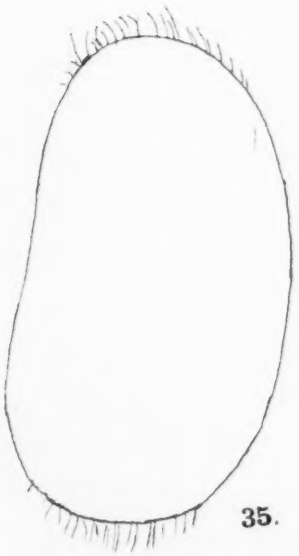

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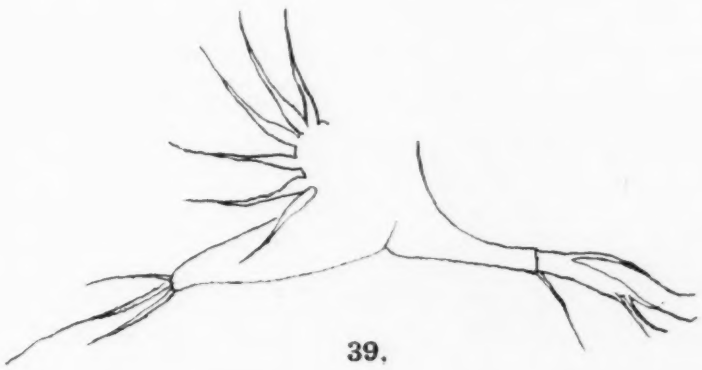

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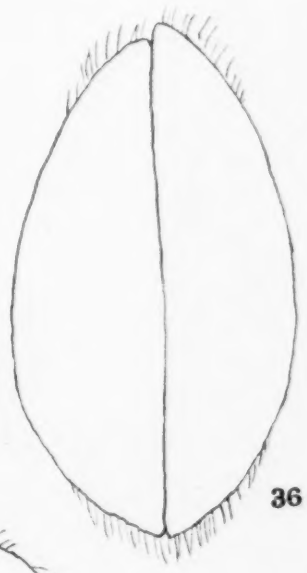

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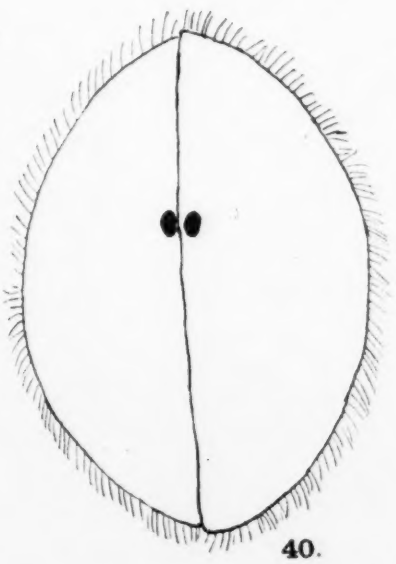

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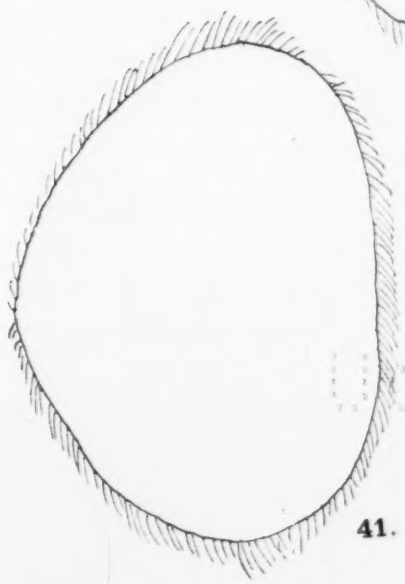

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G. S. BRADY *del ad nat.*

A NEW ANOPHELINE FROM THE FEDERATED MALAY STATES

BY

MALCOLM WATSON, M.D., D.P.H.,

CHIEF MEDICAL OFFICER, KLANG, FEDERATED MALAY STATES.

(*Received for publication 21 June, 1910*)

MYZOMYIA AURIROSTRIS, n. sp.

A small brown anopheline, with white apex to palpi—two broad white bands separated by slightly smaller black bands, and narrow white band near the base—legs unbanded. Proboscis banded. Wings with five costal spots and numerous other spots on the wing field.

Head ♀. Dark brown. Scales. Passing forward between the eyes there is a tuft of long white hairs (among which are to be seen narrow curved scales much as on the wings), and also about half a dozen long dark or brown hairs. Among the roots of these hairs, some white long narrow curved scales are seen. They do not extend either backwards or far from the middle line. Behind these are white upright shallow fork scales, and behind these again black fork scales. Four dark brown hairs project over the lateral region of the eyes.

Thorax. Brown. Dorsum brown, with long brown hairs; projecting over nape some narrow white curved scales. Elsewhere hairy.

Prothoracic lobes brown. Tuft of white narrow-curved and a few blunt scales projecting from anterior part. Elsewhere covered with hairs.

Abdomen. Brown, covered by numerous long brown hairs. Halteres golden scaled.

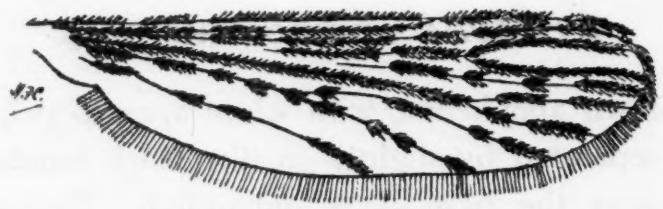
Legs. All brown scaled throughout; ungues, small, equal, simple.

Proboscis. Basal half black scaled. Apical half golden scaled, except for a small but quite definite ring of black scales a short distance from the apex. The apex is golden.*

Palpi. Black, with *four* white bands. The first band consist of a small but definite band of scales at the apex of the first joint. The apex of the last joint is white scaled, with a few white hairs projecting beyond the end. Then follow alternately a narrow black band, a broad white band, a slightly smaller black band, a broad white band, and the remainder of the palpi to the first joint black.

Antennae. Brown.

Scutellum. Brown, with a single row of long dark hairs; metanotum brown, nude.



Wing of *Myzomyia aurirostris*, n. sp.

Wings. Costa, black, broken by five gold spots—two in the basal half of the wing, of which only the outer involves the subcosta and first vein. In the outer half the spot at the junction of the costa and subcosta involves the first vein—the spot of which, however, begins almost where the costal spot ends, and extends towards the apex. It is equal in length to the costal spot. Equidistant between this and the apex is a spot involving the first vein, and at the junction of the first vein and costa there is also a golden spot involving the fringe and a small portion of the first vein and upper branch of second. The first vein, in addition to the above spots, has one just beyond the second costal spot. In one specimen a few golden scales are seen on the costa, not sufficient in numbers to constitute a definite spot, and only involving one side of the vein. On second long vein there are a few golden scales at the origin, a few at the fork, and a few half-way between these two. The third vein begins in a yellow spot, and the rest of the vein consists of four black and three yellow spots alternately—the black spot touching the fringe being longest of all. The fourth vein is black scaled to its fork, except for a few yellow scales

*In the type specimen furnished by the author the integument at the tip is blackish in colour, and the few scales which remain are pale yellowish.—Ed.

opposite the origin of the third vein. At the fork there is a pronounced yellow spot, which extends down each branch, on each of which there is another small yellow spot. At the junction of the branches of the fourth and fifth veins, a few golden scales are seen. The fringe is black even at these points. The fifth vein throughout is alternately yellow and black. Between the origin and the fork there are three yellow spots; one yellow spot involves both branches, but mainly the upper; in addition, there are three yellow spots on each branch in addition to the yellow scales at the junction with the fringe. On first two-thirds of the sixth vein there are three yellow spots. The remainder of the vein is black scaled, with a few scattered golden scales about the middle. Except at the junction of the first vein, the fringe is black throughout. Wing scales long, narrow, lanceolate.

Observations. Bred from two larvae, taken from a hole in the ground 1½ft. wide and 2ft. deep in the jungle next to Merton Estate, Klang, December, 1909.

Table Showing the Chief Differences between *Myzomyia albirostris*, *M. thorntonii*, and *M. aurirostris*

	M. albirostris	*M. thorntonii*	*M. aurirostris*
Palpi	Two broad apical bands and one narrow basal one	Three broad apical bands and a narrow basal band	As in *M. thorntonii*
Proboscis	Apical half pale scaled	Apical half light scaled with a narrow brown band near the apex	As in *M. thorntonii*
Wing Fringe	Pale spots at each vein junction	Mottled—the light spots occurring for the most part at the apices of the veins, long and short scales to the 5th and 6th distinctly yellow	Black
Legs	Unspotted, brown	Spotted and banded	Unspotted, brown
Prothoracic lobes ...	—	Brown flat scales	Tuft of white narrow curved scales from anterior part—elsewhere hairs

ON THE OCCURRENCE OF SCHIZOGONY IN AN AVIAN LEUCOCYTOZOÖN, *L. LOVATI*, PARASITIC IN THE RED GROUSE, *LAGOPUS SCOTICUS*

BY

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(*Received for publication 22nd June, 1910*)

PLATE XXI

Although the Parasitic Protoza are much studied at present, there is no doubt that more facts are required about the life-cycle of many parasites of whose history certain stages are well known. Among such parasites are the avian *Leucocytozoa*.

During the past two years I have spent some of my time as Protozoologist to the Grouse Disease Inquiry, in the investigation of the Protozoa parasitic in the grouse. One, at least, of the protozoal parasites of the grouse, *Eimeria (Coccidium) avium*, is the cause of a rapid and fatal disease among grouse chicks. Among the parasites present in some apparently healthy grouse was a Leucocytozoön, *L. lovati*, first recorded by Seligmann and Sambon in a short note in 1907. My investigation of *L. lovati* led to the elucidation of another phase (schizogony) in its life history.

The host cells of the avian *Leucocytozoa* may be either mononuclear leucocytes or erythroblasts, or both. The exact nature of the host cell of *Leucocytozoa*, which is somewhat controversial, will not be discussed in this paper. Concerning the *Leucocytozoa* themselves, gametocytes, both male and female, have been described from the blood of the host. The male gametocyte of *L. lovati* (Pl. XXI, fig. 1) possesses hyaline, pale-staining cytoplasm, and a rather large nucleus containing a number of chromatin granules. The female gametocyte (fig. 2) has a deeply staining granular and

somewhat alveolar cytoplasm, with a central nucleus containing a karyosome. The exact delimitation of the nucleus of a *Leucocytozoön* within its host cell is often difficult.

In the specimens of *L. lovati* that I examined, the microgametocytes were found to measure from 13μ to 17μ by 6μ to 12μ, while the macrogametocytes were 14μ to 20μ by 10μ to 16μ.

Till recently, the gametocytes and gametes only of avian *Leucocytozoa* were known. However, parasites somewhat intermediate in type between the male and female gametocytes occur, though such intermediate forms are not at all common in the peripheral blood of the host. The intermediate forms in the case of *L. lovati* may be seen in the blood of the spleen of the grouse, though they may be easily overlooked.

In the summer of 1909, I examined two fresh grouse in whose peripheral and heart blood living *Leucocytozoa* were seen. The internal organs were immediately examined after the death of the birds. In stained preparations of the spleens of the two birds, small vermicular forms were encountered, both free and just entered into their host cells. Careful search showed that in the spleen were many full-grown *Leucocytozoa*, some of which exhibited no very definite sexual differentiation. While the gametocytes encountered in the blood often produced marked elongation of the host cells, the deformation in the case of the special splenic forms was slight (fig. 3), and the host cells seemed almost entirely absorbed (fig. 4). These parasites are the schizonts, which are slightly smaller than the gametocytes, and measure 11μ to 14μ by 8μ to 11μ. The cytoplasm of the oval schizonts becomes somewhat concentrated while they are uninucleate (fig. 4). The character of the nucleus of the schizont approaches that of the microgametocyte, while the general cytoplasm resembles that of the macrogametocyte, but differs from it in being less alveolar and possessing smaller granules.

Nuclear multiplication occurs, apparently, by a series of rapid binary fissions, and the daughter nuclei, some of which may remain united (fig. 5) for a short time, ultimately migrate towards the periphery. Segmentation of the cytoplasm around the small nuclei occurs (fig. 6), and the result is that some twelve to twenty merozoites (fig. 7) are produced. Each merozoite is a small, vermicular, somewhat curved organism, capable of active movement. The merozoites measure 7μ

to 8μ by 1μ to 1·5μ. Some residual protoplasm is found in the remains of the schizont after the merozoite formation is completed. The merozoites escape (fig. 8) from the tenuous envelope of the schizont and become free-living forms (fig. 9) in the blood of the spleen. But their free stage of existence is of short duration. Leucocytes or immature erythrocytes are encountered, and the young merozoites penetrate the host cells and enter upon the trophic phase of their existence, ultimately differentiating either as sexual individuals or as schizonts.

The occurrence of schizogony in *L. lovati* is very difficult to demonstrate. Though many infected birds may be examined, schizogony may not be detected, for the period of schizogony may not be attained, and also this form of multiplication is passed through with remarkable rapidity, so that merozoites may be formed and dispersed before the investigator has had time to examine the material available. Rapidity of schizogony is also found in such parasites as *Coccidia*, where often it is not easy to obtain preparations exhibiting schizogony, while some gametocytes can usually be obtained in any preparation from an infected host. In connection with schizogony of *Leucocytozoa*, great interest attaches to the discovery of Mathis and Léger of periodic increases in the number of the gametocytes of *L. caullery*i in the peripheral blood of its host, the domestic fowl of Tonkin, for which increase no explanation has hitherto been afforded. It seems to me that during the intervals between successive crops of parasites, asexual multiplication probably occurs. As in *L. lovati* schizogony occurs in an internal organ, namely, the spleen, it is suggestive that a similar condition prevails in *L. caullery*i; and as in other Protoza, the schizogony culminates in sexual differentiation, so in *L. caullery*i it seems likely that a similar sequence may occur.

It may be added that preparations of bone marrow of the infected birds did not furnish schizonts, though it is possible that schizogony might be found to occur therein, if abundance of material were available for research. *L. lovati* did not appear to be very harmful to the grouse investigated, and in the two cases in which schizogony occurred, no other protozoan parasite was present in the blood of the grouse, all of whose organs were minutely examined.

L. lovati may be transmitted from grouse to grouse by the agency of the grouse-fly, *Ornithomyia lagopodis*, for vermicules devoid of

melanin pigment have been found in the gut of the fly. But, as with most blood parasites, further work is necessary with regard to the exact mode of transmission of protozoal parasites from host to host.

The avian *Leucocytozoa*, as exemplified by *L. lovati*, in which schizogony is now shown to occur, are, then, typical members of the *Haemosporidia*, allied to the malarial parasites.

In conclusion, while the method of schizogony in *L. lovati* is as indicated in this paper, it does not follow that the multiplicative processes of all avian *Leucocytozoa* are on the same lines, though I think that it is probable that in all these avian blood parasites, the period of schizogony is a short one, and that much patient investigation under the most varied conditions will be necessary to establish schizogony in most members of the avian *Leucocytozoa*.

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EXPLANATION OF PLATE XXI

The figures are slightly diagrammatic. Magnification of figs. 1 and 2 about 1,800 diameters; of figs. 3 to 9 about 2,000 diameters.

Figs. 1 and 2. Diagrams of male and female gametocytes of *Leucocytozoön lovati*. The male gametocyte (fig. 1) is usually slightly smaller than the female (fig. 2), and its cytoplasm is hyaline. Chromatoid granules often occur at the ends of the oval or round parasites. The host cell has become spindle-shaped in each case, with its nucleus pushed to one side by the parasite.

Figs. 3 to 9. Diagrams of schizogony of *Leucocytozoön lovati*, as seen in smears of the spleen of the avian host.

Fig. 3. Schizont, with remains of the host cell at either pole and host-cell nucleus to one side.

Fig. 4. Uninucleate schizont, with host cell almost entirely absorbed except for slight remains of the host-cell nucleus to one side.

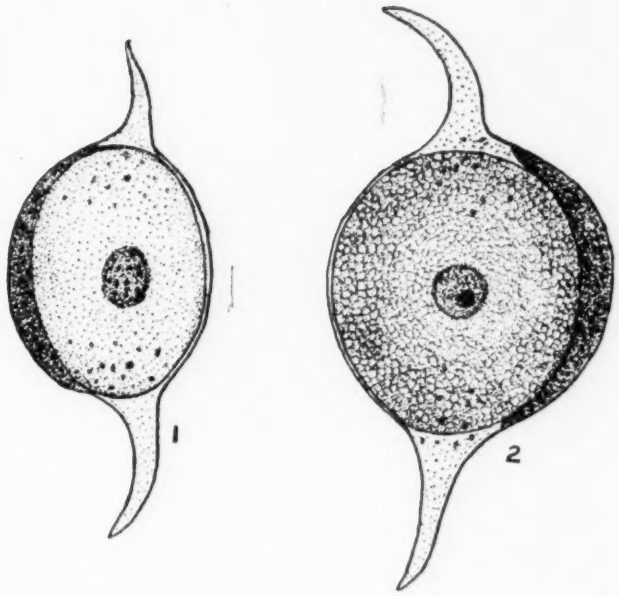
Fig. 5. Schizont, showing nuclear multiplication. Very slight remains of host-cell nucleus on the right side.

Fig. 6. Schizont in process of formation of merozoites.

Fig. 7. Schizont showing merozoites differentiated within.

Fig. 8. Merozoites beginning to escape from schizont.

Fig. 9. Group of free merozoites.



SCHIZOGONY OF LEUCOCYTOZOON LOVATI.

P. P. Press, imp.

[H. B. Fantham, del.

A CASE OF SLEEPING SICKNESS STUDIED BY PRECISE ENUMERATIVE METHODS: REGULAR PERIODICAL INCREASE OF THE PARASITES DISCLOSED

BY

PROFESSOR MAJOR RONALD ROSS, F.R.S.,

AND

DAVID THOMSON, M.B., CH.B., D.P.H.

(*Read before the Royal Society, 16 June, 1910*)

(*Received for publication* 5 *July*, 1910)

PREFATORY NOTE BY R. ROSS

For a long time it has appeared to me that much light might be thrown on infectious diseases, immunity, and treatment, by more exact enumeration of the infecting organisms, and that we might even be able ultimately to apply mathematical reasoning to the study of these subjects. In 1903* I elaborated a method of blood examination, called the thick-film process, which enables us to detect small organisms in the blood about twenty times more easily than in ordinary preparations; but for the lack of the necessary assistance I was long unable to apply the method to the laborious enumeration of such organisms. Recently, however, the Advisory Committee for the Tropical Diseases Research Fund has placed considerable funds at the disposal of the Liverpool School of Tropical Medicine for the study of cases in the tropical ward at the Royal Southern Hospital, Liverpool, with the result that the investigations referred to were commenced by Dr. David Thomson and myself from the beginning of this year. As I expected, methodical counting of the parasites has at once verified or disclosed several facts of importance in connection with malaria and trypanosomiasis. We now limit ourselves to a brief description of the remarkable periodical increase of *Trypanosoma*

* Lancet, 10th Jan., 1903, and Thompson-Yates Reports, Vol. V, part I, 1903.

gambiense revealed by careful daily counting in a case in my clinic (the case, which is still under treatment, will be fully reported elsewhere).

W. A., male, aged 26, a strong young man, born in Northumberland, was infected in North-East Rhodesia in September, 1909, the trypanosomes being found in his blood in Africa on November 17. On coming to Liverpool for treatment he was admitted into the Southern Hospital on December 4. From then until February 16 (seventy-three days) the number of trypanosomes in his blood was estimated only by the rough methods in common use—that is, by the proportion of trypanosomes to red cells, or leucocytes, or to 'fields' of the microscope examined. These methods are obviously open to such great error that they can scarcely be depended upon to indicate any but very large differences in the numbers of objects counted. During the seventy-three days forty-six counts were made, but on several occasions none was attempted for three or four days in succession; so that, even if the methods of counting employed had been more accurate, sudden fluctuations might easily have been missed. Hence, as was to be expected, the graph during this period is very irregular and almost worthless. On admission on December 4 the patient was reported to contain about 6,000 trypanosomes per cubic millimetre of blood, and large numbers, amounting to about 3,000 per cubic millimetre, were found on December 17 and 28, and on January 16. All this time the patient was given the usual treatment with atoxyl and mercury, and received altogether ten doses of 2 to 4 gr. of the former. Nevertheless, the parasites never fell below about 200 per cubic millimetre in number, as roughly estimated.

It was then found, however, that atoxyl was injuring the patient's sight—as sometimes happens—and other treatment was substituted. At the same time we elaborated a much more correct method of counting all the parasites in measured quantities ($\frac{1}{4}$ to 1 c.mm.) of blood taken in thick film, and from February 16 onward the patient's trypanosomes were estimated daily by this method by one of us (D.T.). The attached chart gives the remarkable graph obtained up to the present (April 30).

The numbers of trypanosomes found were scrupulously recorded, and the smoothness and regularity of the graph suggest that there was no very great error of observation. The blood was taken every day at about 10 a.m., but on April 5 and 6 several counts were made daily.

It will be seen that between February 16 and April 30 (seventy-three days) there were eleven rises in the number of the parasites. Up to April 7 there were seven rises, at intervals of seven or eight days. During this period the patient was given no atoxyl, but was treated with large doses of quinine (30 to 40 gr.) daily, with frequent doses of methylene blue and with trypan red on March 17, 18, 19, and 20.

On April 5 it was decided to administer atoxyl again, as shown on the chart, together with mercury and other treatment.

The temperatures were taken by the sister of the ward, and it will be seen that there has always been a tendency to a slight rise in temperature concurrent with the rise in the number of parasites —the two curves thus confirming each other. Only the maximum and minimum temperatures are entered in the accompanying chart.

The great regularity of the rises can scarcely be compatible with a mere chance distribution. It will also be observed that the rises were of two kinds—namely, high rises and low rises—and that the two kinds alternated with regular periodicity until April 18, at which point the cycle appears to have become distorted—probably in consequence of the treatment. The regularity of alternation of the high and low rises is so well marked as to recall the picture of a double tertian malaria, and to suggest that two independent sets of parasites may exist in the patient, just as often happens in malaria.

The large dose of atoxyl given on April 5 seems to have had no effect whatever on the following rise; but the succeeding rises were apparently modified for some reason. The value of the enumerative method for therapeutical research is obvious.

Of course, many other facts in connection with the case have been recorded, and parallel work is being done on sub-inoculated animals and on the parasites. It is therefore inadvisable to attempt at present any discussion of the many interesting theoretical questions which arise.

We are much indebted to the Director of the Sleeping Sickness Bureau (Dr. Bagshawe) for having given us references to the literature on the subject of such fluctuations. In the original case of Dutton and Ford it was noted that the parasites varied in numbers, and that a parallel rise in the patient's temperature occurred. Manson and Daniels* chart the number of parasites compared with 500 leucocytes; but the error of this method is very large and their graph is quite irregular. They abandoned counts in measured quantities of blood as 'unreliable.' Thomas and Breinl† showed that in three cases of sleeping sickness the numbers of trypanosomes found in 'fresh cover-slip preparations' varied irregularly from time to time. Koch, Beck, and Kleine (1909) remark on the irregularity of the appearance of *T. gambiense* in African natives, and state the parasites are present for two to five days and absent for two to three weeks. Salvin-Moore and Breinl‡ show a graph with two undulations and a final premortal rise in two heavily infected rats, and give a detailed description of corresponding changes in the parasites. Apparently, hitherto, only irregular variations in the numbers of the parasites seem to have been recognised; probably the large error due to inadequate methods of counting has disguised the regular periodicity of the variation shown by more exact counts in the eleven successive undulations observed in our case.

We should add that our methods enable us to detect parasites when they are in numbers so small that their detection by the ordinary methods would be exceedingly laborious. Hence if our case had been studied by the ordinary methods, probably only the crests of the rises would have been visible in the chart, and it would have been said that the parasites had disappeared in the intervals.

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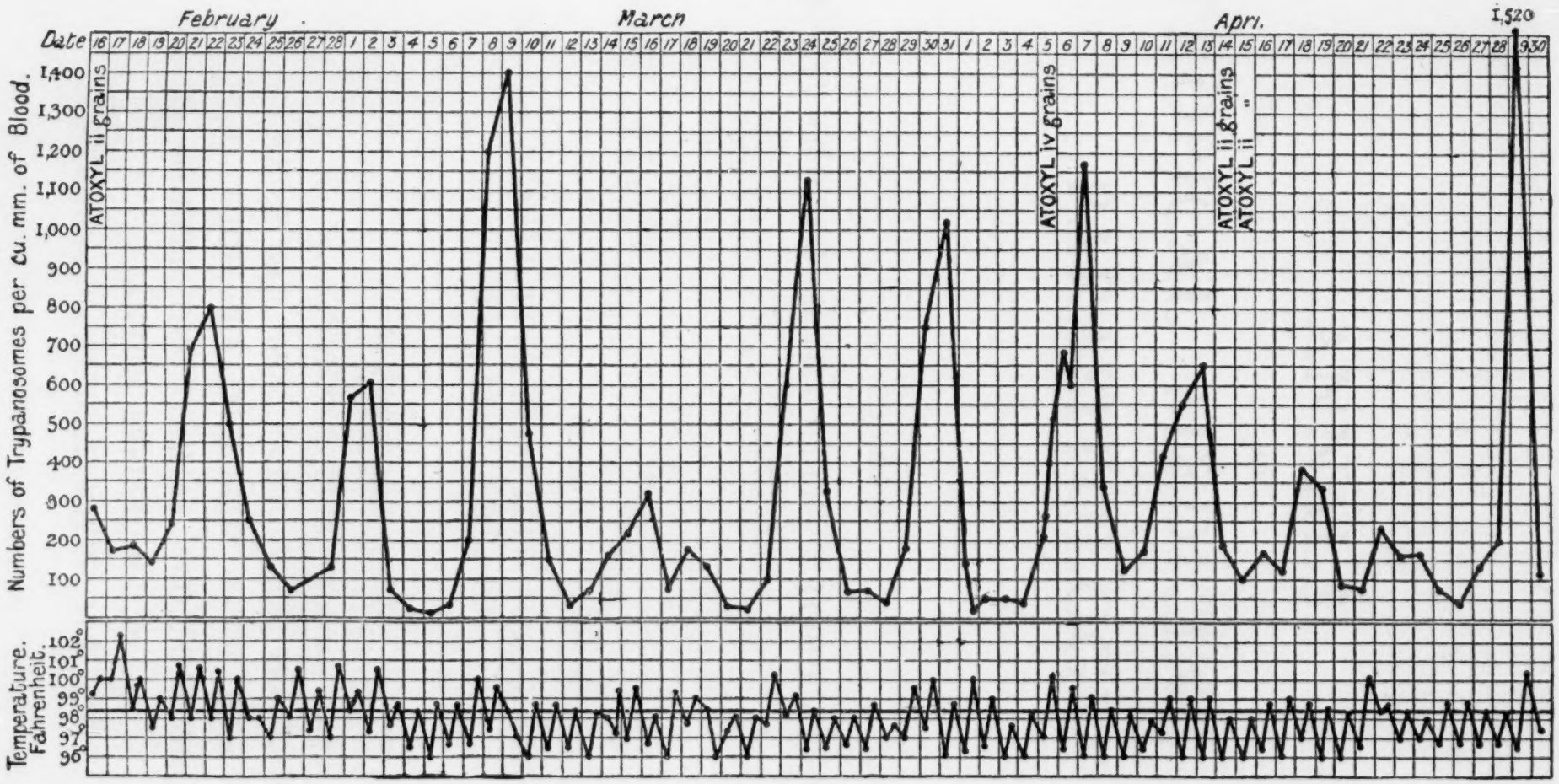
* Brit. Med. Journ. May 30, 1903.

† Memoirs of the Liverpool School of Trop. Med. Vol. XVI, 1905.

‡ Annals of Trop. Med. and Parasit. Vol. I, No. 3, 1907.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March
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ATOXYL ii grains
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ATOXYL ii grains
ATOXYL ii "
Numbers of Trypanosomes per cu. mm. of Blood.
1,400
1,300
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Temperature. Fahrenheit.
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